QK1 .F36

THE FERN GAZETTE

EDITORS:
M. GIBBY & A. LEONARD

VOLUME 17 PARTS 6, 7 & 8 2006

THE FERN GAZETTE is a journal of the British Pteridological Society and contains peer-reviewed papers on all aspects of pteridology.

Manuscripts may be submitted, and books etc. sent for review, to: Prof. M. Gibby, Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, EH3 5LR, UK
Telephone: 0131-248-2973 E-mail: FernGazette@eBPS.org.uk

Instructions for authors are on page 368 of this volume and also available at http://www.eBPS.org.uk

Copyright © 2006 British Pteridological Society. All rights reserved. No part of this publication may be reproduced in any material form (including photocopying or storing it in any medium by electronic means) without the permission of the British Pteridological Society.

THE FERN GAZETTE Volume 17 Part 5 was published on 20th September 2006

Published by THE BRITISH PTERIDOLOGICAL SOCIETY
c/o Department of Botany,
The Natural History Museum, London SW7 5BD, UK

Printed by Bishops Printers Limited
Fitzherbert Road, Farlington, Portsmouth, PO6 1RU, UK
www.bishops.co.uk

Cover design by Hazel Sims

A NOTE TO SUBSCRIBERS AND MEMBERS

Recipients of the Fern Gazette will have noted that publication of the journal appears to have fallen behind schedule over the last few years. Volume 17 was due to have been completed by the publication of Parts 7 and 8 during 2006, whereas only Parts 1 to 5 have been issued until now.

This apparent reduction in output is misleading. We have in fact produced more than the scheduled number of pages in Volume 17 and also previously in Volume 16 but they have been issued in over-size parts. We normally issue two parts per year, each of approximately 40 pages, and 8 parts (i.e. approximately 320 pages) per volume. Volume 14 contained 312 pages and Volume 15, 308 pages. However, Volume 16, parts 1 to 8, contained 481 pages, and already in Parts 1 to 5 of Volume 17 we have issued 312 pages.

The main reason for issuing over-size parts during the last 5 years was the need to publish papers from two Symposia, "Fern Flora Worldwide: Threats and Responses" (2001) and "Ferns for the 21st Century" (2004), as they became available and without undue delay. For the same reason, the parts were not published at regular 6-monthly intervals.

Now that all the symposium papers have been published, future twice-yearly issues will return to the usual size of approximately 40 pages.

In order to complete volume 17 and commence Volume 18 in 2007 as scheduled, this issue has been numbered as "Parts 6,7 and 8". Volume 17 will thus have a total of 368 pages.

We apologise if the recent variation in the size and irregular appearance of parts has caused any difficulties but hope you will now be reassured that you have received the full quota of pages, and more, for volumes 16 and 17.

In this part we are introducing the first of what we plan to be a series of mini-review articles for the Fern Gazette. Our first invited review article is on pteridophyte cell walls, by Dr Zoe Popper. We welcome offers of other review papers of that would be of interest to our readership.

M. Gibby, A. Leonard (Editors)
November, 2006

MISSOURI BOTANICAL
DEC 2 7 2006
GARDEN LIBRARY

BOOK REVIEW

THE FIRST BOTANICAL COLLECTORS IN NEPAL – The Fern collections of Hamilton, Gardner and Wallich – lost herbaria, a lost botanist, lost letters and lost books somewhat rediscovered. Fraser-Jenkins, C.R. 2006. Hardback with dust wrapper. vi + 106 pp., 12 pls (colour). ISBN 81-211-506-4. Bishen Singh Mahendra Pal Singh, Dehra Dun. 30 Euros ($40).

The title of this short book and its first subtitle summarise the subjects covered, while the second subtitle serves to convey something of its flavour. This is an important contribution to existing literature on the botanical history of the Indian subcontinent, and is of far wider interest than the merely pteridological. In its personal and somewhat idiosyncratic style it is reminiscent of writings of an earlier age, which, while not without its own dangers, comes as a welcome change from the more arid style typical of run-of-the-mill scientific and historical works.

Fraser-Jenkins, who is based in Kathmandu, is well known for his work on Asian ferns, a combination that has led him to unscramble successfully the complexities of the early collections of Nepalese plants and publications thereon. Anyone who has worked on these matters will be aware of the difficulties of the 'Wallich Herbarium' (actually the herbarium of the Hon. East India Company), its catalogue – Wallich's *Numerical List* with its *nomina nuda*, and the frustrations of trying to typify names published in David Don's *Prodromus Florae Nepalensis* (which Fraser-Jenkins convincingly attributes to 1824 rather than the more usually cited 1825). The author has applied great skill – and a steely determination – in untangling these knotty problems, and the results are presented here in all their rich detail: botanical and biographical. Fascinating information is provided of the earliest Western botanists to work in Nepal: Francis Buchanan (later Hamilton), the Hon. Edward Gardner, Brian Houghton Hodgson, William Jack and Nathaniel Wallich. That for Gardner, the 'lost botanist' of the title, is particularly welcome, and he emerges as a fascinating character in the 'White Mughal' tradition (a tradition that appears to be alive and well, and living in Kathmandu!).

The story of Don's *Prodromus* is given in detail, and the reasons for the opprobrium heaped on it by John Lindley and Wallich shown to be unfair to Don, and more to do with the circumstances of its commissioning by A.B. Lambert. Fully explained is the Buchanan and 'Wallich' material on which Don worked, the latter being collected by Gardner several years before Wallich's own first and only visit to the country in 1820–1. Useful notes are given on how to typify Don's names, and appropriate warnings not to use later, numbered, Nepalese specimens from the EIC herbarium.

For this work a truly impressive range of sources, both printed and archival, has been used – a major achievement given the author's lack of an official position, and his base in Nepal, which allows only occasional visits to Western libraries and herbaria. This tenacity of purpose has reaped huge rewards in major discoveries such as the manuscript of Thomas Moore's *Index Filicum* in a well known library on the banks of the Thames, and a find of much wider significance on the banks of the Hooghly. The latter is Wallich's entire incoming correspondence tragically repatriated to Calcutta by Kew in 1887, and the manuscript of his unpublished 'Filicologia Nepalensis' of 1821. Fraser-Jenkins draws attention to the perilous state of this archive and it is fervently to

continued on page 350

THE CELL WALLS OF PTERIDOPHYTES AND OTHER GREEN PLANTS – A REVIEW

Z.A. POPPER

Current address: The Department of Botany, The Martin Ryan Institute,
National University of Ireland Galway, Ireland
(Tel.: +353 91 49 5431. fax: +353 91 49 4543. Email: zoe.popper@nuigalway.ie)
Previous address: The Complex Carbohydrate Research Centre,
University of Georgia, 315 Riverbend Road, Athens, GA 30602, USA.

Key words: cell wall, evolution, terrestrialisation, vascularisation, xyloglucan

ABSTRACT

The cell wall is one of the defining characteristics of plants and is a fundamental component in normal growth and development. Cell wall composition is a potentially valuable source of phylogenetic information as notable similarities and differences exist between and within major embryophyte groups. In particular, there is a pronounced chemical demarcation between the eusporangiate pteridophytes (high mannan, low tannin) and the leptosporangiate pteridophytes (low mannan, high tannin). The results of recent biochemical and immunocytochemical investigations have shown that changes in cell wall composition accompanied the bryophyte–lycopodiophyte and eusporangiate–leptosporangiate transitions.

CELL WALL FUNCTION

The earliest plants existed in an aqueous environment and their cell walls evolved in large part as one of the strategies to counteract the associated osmotic stress (Gerhart & Kirshner, 1997). The cellulose-rich cell wall is one of the defining characteristics of plants, most of which now inhabit terrestrial environments. When the plant cell wall was first described by the microscopist Robert Hooke in the seventeenth century it was considered to be an inert skeleton. However, these walls are now known to have numerous biological roles, including the regulation of cell expansion, the control of tissue cohesion, defence against microbial pathogens, and ion exchange, and are a source of biologically active oligosaccharides (Goldberg *et al.,* 1994; Brett & Waldron, 1996; Cassab, 1998; Dumville & Fry, 1999; Fry, 1999; Côté & Hahn, 1994; Côté *et al.*, 1998). The cell wall is a dynamic structure that is continually modified by enzyme action during growth, development, environmental stress and infection (Cassab 1998).

Stebbins (1992) suggested that changes in cell wall composition were involved in bryophyte diversification and had a role in the evolution of leptosporangiate ferns from their eusporangiate ferns ancestors. The new environmental challenges experienced during the colonization of land and those experienced during the development of the tracheophyte and leptosporangiate conditions may have driven rapid evolution of cell walls and led to the differences in wall composition between groups of extant land plants that will be discussed in this review.

TYPES OF CELL WALL

Cell walls consist of three types of layers: the middle lamella, the primary cell wall and

the secondary cell wall. The middle lamella is deposited soon after mitosis and creates a boundary between the two daughter nuclei. The location of the new wall is directed, in charophycean algae and land plants, by the phragmoplast, a cluster of microtubules (Pickett-Heaps & Northcote, 1966; Marchant & Pickett-Heaps, 1973; Brown & Lemmon, 1993). The primary cell wall, typically 0.1–10 µm thick, is deposited once the cell plate is complete and continues to be deposited whilst the cell is growing and expanding. The primary cell wall defines cell shape and thereby contributes to the structural integrity of the entire plant. At maturity some common cell types (parenchyma and collenchyma) frequently have only a primary cell wall.

The fixed, immobile nature of plant cells and tissues means that the plane of cell division and the sites of cell expansion are closely regulated and exert a strong influence on subsequent plant morphology (Fowler & Quatrano, 1997). Cellulose microfibril orientation controls the direction of cell elongation (Saxena & Brown, 2005); therefore mutations that resulted in changes in the mechanisms of early cell wall deposition, particularly those concerning cellulose, are likely to have had significant effects on plant evolution. Niklas (2005) has hypothesised lateral transfer of cellulose synthase genes across diverse prokaryotic and eukaryotic species because of similarities in cellulose synthesis mechanisms (Giddings *et al.*, 1980; Wada & Staehelin, 1981; Murata & Wada, 1989). Control over the orientation of new cell wall divisions may exert some influence over the direction of plant growth. Light has been shown to exert control over the positioning of new cell walls in apical cells of fern gametophytes (Racusen, 2002) in such a way as to cause two dimensional growth. A plant will then exhibit an upward growth habit in addition to growing flat on the substrate. Light availability was probably an important environmental stimulus driving evolution of vertical plant growth and therefore vascular plants.

A secondary cell wall, if present, is laid down internally to the primary cell wall at the onset of differentiation, once cell growth has ceased. Secondary cell wall composition and ultrastructure in spermatophytes varies from one cell type to another as well as between plant species. This variability may reflect specific cell function. For example, many secondary cell walls, particularly xylem cells, contain lignin which increases wall strength.

CELL WALL COMPOSITION

Analytical methods used in cell wall studies

Cell wall composition has been determined using numerous analytical methods. The glycosyl residue composition of a cell wall polysaccharide is typically obtained after acid hydrolysis. The released glycoses are then identified using paper chromatography, thin-layer chromatography, high pressure liquid chromatography or gas chromatography (Fry, 2000). Numerous chemical and enzymic methods have been developed to generate oligosaccharide fragments from specific polysaccharides that can be structurally characterised by nuclear magnetic resonance spectroscopy and mass spectrometry. Immunocytological methods are becoming increasingly valuable tools for wall analysis with the increased availability of polysaccharide-specific monoclonal antibodies (Knox, 1997; Willats *et al.*, 1998, 2000; http://cell.ccrc.uga.edu/~mao/wallmab/Antibodies/antib.htm), and proteins that contain specific carbohydrate-binding modules (McCartney *et al.*, 2004). Several monoclonal antibodies raised to angiosperm cell wall polysaccharides (Willats *et al.*, 2000; Jones *et al.*, 1997; Puhlmann *et al.*, 1994; Freshour *et al.*, 1996) are able to recognise at least

some epitopes (structures within a molecule which are recognised by antibodies; a macromolecule may contain many distinctly different epitopes) in pteridophyte cell walls (Figure 1) indicating that some of the structures present in angiosperm cell wall polysaccharides are conserved.

Primary cell wall polysaccharides

The primary cell wall is composed of crystalline cellulose microfibrils that are embedded in a gel-like matrix of non-cellulosic polysaccharides and glycoproteins (Fry, 2000). Current primary cell wall models depict a cellulose-hemicellulose network that is formed from cellulose microfibrils that are interconnected by hemicellulosic polysaccharides, such as xyloglucan, mixed-linkage β-glucan or arabinoxylan, forming a cellulose–hemicellulose network (Carpita & Gibeaut, 1993; Mishima *et al.*, 1998). The cellulose–hemicellulose network coexists with a second network that consists of the pectic polysaccharides homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II and a further network of structural glycoproteins.

Detailed structural studies of primary cell walls have only been performed on a limited number of angiosperms and gymnosperms. Nevertheless, it is generally assumed that seed plants have primary cell walls with similar but not identical compositions. The qualitatively predominant monosaccharides present in primary cell wall polysaccharides are D-glucose (Glc), D-galactose (Gal), D-mannose (Man), D-xylose (Xyl), L-arabinose (Ara), L-fucose (Fuc), L-rhamnose (Rha), and D-galacturonic acid (GalA) (Albersheim, 1976; McNeil et al, 1984; Fry, 2000). The primary cell walls of gramineous monocots typically contain more Xyl and less GalA, Gal and Fuc (Burke *et al.*, 1974; Carpita, 1996) than other angiosperms whereas gymnosperm primary cell walls are similar in composition to those of dicotyledonous angiosperms but contain more Man residues (Edashige & Ishii, 1996; Thomas *et al.*, 1987; Popper & Fry, 2004).

Additional variation of cell wall composition exists at the polysaccharide level. Mixed-linkage glucans appear to occur only in gramineous monocots and closely related members of the Poales (Smith & Harris, 1999). Pectic polysaccharides are major components of the primary cell walls of dicots, non-gramineous monocots, and gymnosperms. Recent studies suggest that these polysaccharides are also abundant in fern cell walls (Popper & Fry, 2004; Matsunaga *et al.*, 2004). Xyloglucan, a hemicellulosic polysaccharide, is present in the cell walls of bryophyte gametophytes (Kremer *et al.,* 2004), pteridophytes, lycopodiophytes, gymnosperms, and angiosperms, but has not been detected in the cell walls of charophycean green algae (Popper & Fry, 2003). Similarly, hydroxyproline (Hyp), a major component of cell wall glycoproteins and proteoglycans, is present in embryophyte but not charophyte walls (Gotteli & Cleland, 1968). Thus, the appearance of xyloglucan and Hyp-rich proteins in primary cell walls is likely to have occurred after the divergence of charophytes and embryophytes from their last common ancestor (Niklas, 2005).

Pteridophytes, gymnosperms and angiosperms form a well-supported monophyletic group, the tracheophytes (vascular plants) which originated approximately 420 million years ago (Judd *et al.*, 1999). The primary cell walls of extant pteridophytes (= non-seed vascular plants, including ferns, horsetails and club-mosses; Pryer *et al.*, 2001) have not been studied in detail. However, there is increasing interest in the walls of non-seed plants because an understanding of the structures and functions of their walls may provide insights into the evolution and wall biology of seed plants. For example, in a recent report it was shown that pteridophyte and spermatophyte walls contain

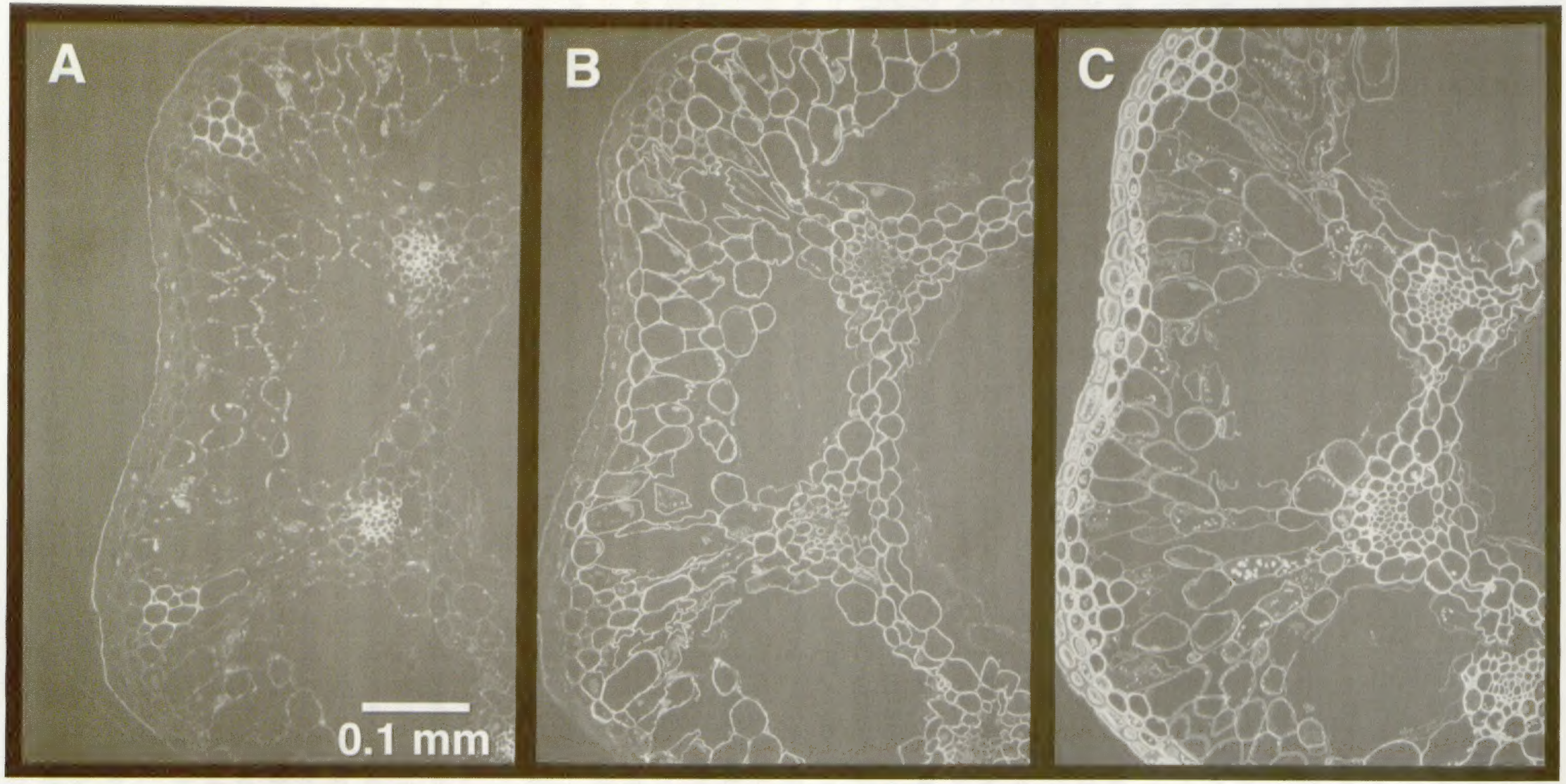


Figure 1. Transverse sections of *Equisetum sp.* stem labelled with the monoclonal antibodies. A: CCRC-M1, which recognises epitopes present in fucosylated xyloglucan; B: LM5, which recognises 1,4-linked β-D-galactan; and C: LM6, which recognises 1,5-linked α-L-arabinan.

comparable amounts of the borate-ester cross-linked pectic polysaccharide referred to as rhamnogalacturonan II. The structure of rhamnogalacturonan II is conserved in the walls of angiosperms, gymnosperms, pteridophytes and lycopodiophytes (Matsunaga *et al.*, 2004). Conclusive evidence for the occurrence of rhamnogalacturonan II in bryophytes is lacking because none of the sugars diagnostic for this polysaccharide were detected in the walls of avascular plants (Matsunaga *et al.*, 2004). It is therefore likely that rhamnogalacturonan II evolved in parallel with vascular plants. The conservation of rhamnogalacturonan II structure is quite remarkable since this polysaccharide contains 12 different glycoses linked together by 20 different glycosidic linkages. It is likely that rhamnogalacturonan II structure is constrained because its cross-linking involves the formation of a borate diester between two specific apiose residues. Recent studies have shown that plants carrying mutations that result in altered rhamnogalacturonan II structure have decreased borate cross-linking of rhamnogalacturonan II and exhibit growth abnormalities (O'Neill *et al.*, 2001, 2003; Ryden *et al.*, 2003).

Numerous morphological gaps exist between bryophytes and lycopodiophytes and lycopodiophytes and pteridophytes (Kenrick & Crane, 1997). These arise largely owing to the fact that extant pteridophytes are the surviving progeny of once diverse and ecologically dominant taxa (Raven, 1993; Kenrick & Crane, 1997). Nevertheless, many protracheophyte fossils have remnants of cell wall tracheary tissues that span some of the morphological gaps between bryophytes, lycopodiophytes and pteridophytes (Boyce *et al.*, 2003).

Extant ferns are divided into two groups: eusporangiate (where the sporangium wall has two or more cell layers) and leptosporangiate (where the sporangium wall has just one cell layer). The majority of extant ferns belong to the monophyletic leptosporangiate group which is also defined by the presence of an annulus, a sporangial stalk, a vertical first zygotic division and a primary xylem with scalariform pits. The eusporangiate condition is generally believed to have diverged earlier and is characteristic of lycopodiophytes, equisetophytes and psilotophytes as well as ferns in the Ophioglossaceae and Marattiaceae. These ferns, are believed to be among the earliest diverging (Smith, 1995; Pryer *et al.*, 1995).

Differences in cell wall composition correlate with emergence of the tracheophytes and divergence of the pteridophytes. Divergence of leptosporangiate and eusporangiate ferns is associated with a decrease in the amounts of mannose in the primary cell wall (Popper & Fry, 2003, 2004). Eusporangiate pteridophyte and bryophyte primary cell walls typically contain more mannose than the primary cell walls of leptosporangiate pteridophytes (Popper & Fry, 2003, 2004). Moreover, proanthocyanidins are associated with primary cell walls from leptosporangiate ferns, gymnosperms and angiosperms, but not eusporangiate pteridophytes (Popper & Fry, 2004; Bate-Smith & Learner, 1954). Leptosporangiate ferns are the earliest diverging plants in which proanthocyanidins start to predominate over flavonols (De Bruyne *et al.*, 1999). It is likely that the production of proanthocyanidins evolved at the same time as the leptosporangiate condition rather than the vascular condition because proanthocyanidins remain important in early diverging angiosperms but their synthesis decreases in more advanced orders. Leptosporangiate ferns appear to have diversified in an environment dominated by seed plants and may therefore have faced selective pressures similar to those experienced by seed plants (Schneider *et al.*, 2004). This may explain why the primary cell walls of leptosporangiate ferns, including *Osmunda*, one

of the earliest diverging extant leptosporangiate ferns (Pryer *et al.*, 2001; Schneider *et al.*, 2004), are more similar to those of seed plants than to those of eusporangiate pteridophytes. Table 1 summarises the differences in cell wall composition found between different land plants.

The lycopodiophytes form a distinctive, basal, monophyletic clade within the eutracheophytes and evolved during the Devonian (408–360 million years ago) (Bateman *et al.,* 1998). The seldom observed monosaccharide residue, 3-*O*-methylgalactose (3-*O*-MeGal) is a quantitatively major component of homosporous and heterosporous lycopodiophytes primary cell walls (Popper & Fry, 2001). It is likely that 3-*O*-MeGal is a component of many lycopodiophyte primary cell wall polysaccharides (including xyloglucan; Malcolm O'Neill, personal communication). The occurrence of this sugar may support the claim of monophyly of lycopodiophytes since little if any 3-*O*-MeGal is present in the primary cell walls of other land plants investigated (Popper & Fry, 2001). The divergence of the lycopodiophytes and the subsequent divergence of the extant homosporous and heterosporous clades appear however to be associated with a decrease in the amounts of *O*-methylated sugar residues. Small amounts of 3-*O*-methylrhamnose (3-*O*-MeRha; trivial name acofriose) have been detected in the primary cell walls of charophytes, bryophytes, pteridophytes and gymnosperms (Popper & Fry, 2003; Matsunaga *et al.*, 2004; Akiyama *et al.*, 1988; Anderson & Munro, 1969). Matsunaga *et al.* (2004) demonstrated that this monosaccharide was present as a component of rhamnogalacturonan II isolated from several pteridophytes. However, no 3-*O*-meRha has been detected in angiosperm primary cell walls, showing that the ability to synthesise this *O*-methylated sugar is not required for land plant survival. The existence of 3-*O*-meRha in a wide range of diverse plants could be explained by the rapid divergence of lineages that occurred during the emergence of the euphyllophytes (Pryer *et al.*, 2001).

Secondary cell wall polysaccharides

Xylans are the quantitatively major cellulose-linking polysaccharides in higher plant secondary cell walls. Typical angiosperm and gymnosperm xylans consist of a 1→4-linked β-D-xylopyranose backbone with a single α-glucuronic acid or 4-*O*-methyl-α-D-glucuronic acid residue attached to the O-2 position of the xylose residues (Shatalov *et al.*, 1999). Xylans with a similar structure have been isolated from secondary cell walls of *Osmunda cinnamomea* (Timell, 1962).

Immunocytological studies using the monoclonal antibodies LM 10 and LM11 that recognise epitopes on xylans have shown that these polysaccharides are present in the secondary cell walls of vascular and mechanical tissues in all extant tracheophytes investigated (McCartney *et al.*, 2005; Carafa *et al.*, 2005). Epitopes recognized by LM11 (substituted xylans and arabinoxylans) are present in specific cell-wall layers in hornwort pseudoelators and spores (Carafa *et al.*, 2005) but the epitopes recognized by LM10 (unsubstituted (1→4)-β-xylans) were absent (Carafa *et al.*, 2005). LM10 and LM11 did not bind to liverwort and moss cell walls (Carafa *et al.*, 2005), suggesting that no detectable amounts of xylan are in the thickened cell walls of these bryophyte groups. The ubiquitous occurrence of xylans in tracheophytes indicates that xylans may have provided a pre-adaptive advantage, occurring in protracheophytes, allowing the evolution of highly efficient vascular and mechanical tissues and enabling the tracheophytes to develop a larger size and to colonize water-limited environments

Table 1: Composition of land plant cell walls.

Plant Group	Monosaccharides		Polysaccharides					Other
	3-*O*-MeRha	3-*O*-MeGal	Xylan	Mannan	Xyloglucan	Rhamnogalacturonan II	Pectin	Tannins
Charophytes	+	–	–	+	–	±	++	–
Hornwort	+	–	+	+	+	±	+++	–
Liverworts and basal mosses	+	–	–	+	+	±	++	–
Advanced mosses	+	–	–	+	+	±	++	–
Homosporous lycopodiophytes	+	+	+	+	+	+	+	–
Heterosporous lycopodiophytes	–	+	+	+	+	+	+	–
Eusporangiate ferns	–	±	+	+	+	+	+	–
Leptosporangiate ferns	–	±	+	±	+	+	+	+
Spermatophytes	–	±	+	±	+	+	+	+

–, Not detectable; ±, trace; +, present at low concentration; ++, present at moderate concentration; +++, present at high concentration.

(Bateman *et al.*, 1998). The immunocytological studies of Carafa *et al.* (2005) suggest that hornworts may be sister to the tracheophytes.

The backbones of galactoglucomannans are composed of alternating (1→4)-linked β-D-mannopyranose and β-D-glucopyranose residues. Some of the mannose is substituted at O-6 by α-D-galactopyranose or β-D-galactopyranose-(1, 2)-α-D-galactopyranose side chains. Galactoglucomannans are major components of the cell walls of the woody tissues of both angiosperms and gymnosperms, and are minor components in the primary cell walls of angiosperm and gymnosperm cambial tissues, in suspension-cultured tobacco cells (Eda *et al.*, 1985), and in secondary cell walls from the stem tissues of the aquatic moss *Fontinalis antipyretica* (Geddes & Wilkie, 1971; 1972) and the fern *Pteridium aquilinum* (Bremner & Wilkie 1971). The solid-state ^{13}C NMR spectra of fibrous material of the silver tree fern, *Cyathea dealbata* contain a weak signal at 102 ppm that may originate from C-1 of a mannose residue in a glucomannan or a mannan (Newman, 1997). The occurrence of galactoglucomannans in secondary cell wall tissues, including those of bryophytes, indicates galactoglucomannans evolved prior to the divergence of tracheophytes. Galactoglucomannans may have played an important role in providing tensile strength in bryophyte secondary cell walls.

Cell wall composition in relation to plant phylogeny

Major transitions in cell wall diversity were mapped against the phylogeny of Kenrick and Crane (1997; Figure 2) because this phylogeny is one of most comprehensive treatments of plant taxa and their closest extant common ancestors, the charophycean green algae. The phylogeny proposed by Pryer *et al.* (2001) is based on vegetative and reproductive morphological/anatomical characters in addition to plastid (*atpB*, *rbcL* and *rps4*) and nuclear (small subunit) DNA sequence data. Pryer *et al.* (2001) place the bryophytes as basal but the relationships to each other and to vascular plants remain unresolved whereas Kenrick and Crane (1997) suggest liverworts to be the earliest evolving among land plants. On the basis of cell wall characters, hornworts may be basal among bryophytes as they (and possibly some charophycean green algae) contain the unusual disaccharide α-D-glucuronosyl-(1→3)-L-galactose (Popper *et al.*, 2003). Cell walls of hornwort also have a high concentration of glucuronic acid; the concentration of glucuronic acid in cell walls of liverworts and mosses is lower than that in hornworts but greater than that of vascular plants (Popper *et al.*, 2003). However, cell wall characters also suggest a close relationship between hornworts and tracheophytes. Immunocytological studies by Carafa *et al.* (2005) show hornworts to be the only bryophytes to contain substituted xylans and arabinoxylans. Among extant taxa, substituted xylans and arabinoxylans are found predominantly within the tracheophytes (Carafa *et al.*, 2005). Mapping further cell wall related characters onto the phylogeny generated by Pryer *et al.* (2001) shows that cell wall characters partially support the three tracheophyte divisions suggested namely (1) lycopodiophytes, (2) seeds plants and (3) a group consisting of equisetophytes, psilotophytes, eusporangiate and leptosporangiate ferns. Pteridophyte cell walls, in common with lycopodiophytes, differ from spermatophytes in being mannose-rich. Lycopodiophytes are supported as being monophyletic primarily by the presence of 3-*O*-methylgalactose which is absent or present at much lower concentration in other tracheophytes. However, spermatophyte and filicophyte cell wall preparations are both associated with tannins (Popper & Fry, 2004) which would split the group containing equisetophytes,

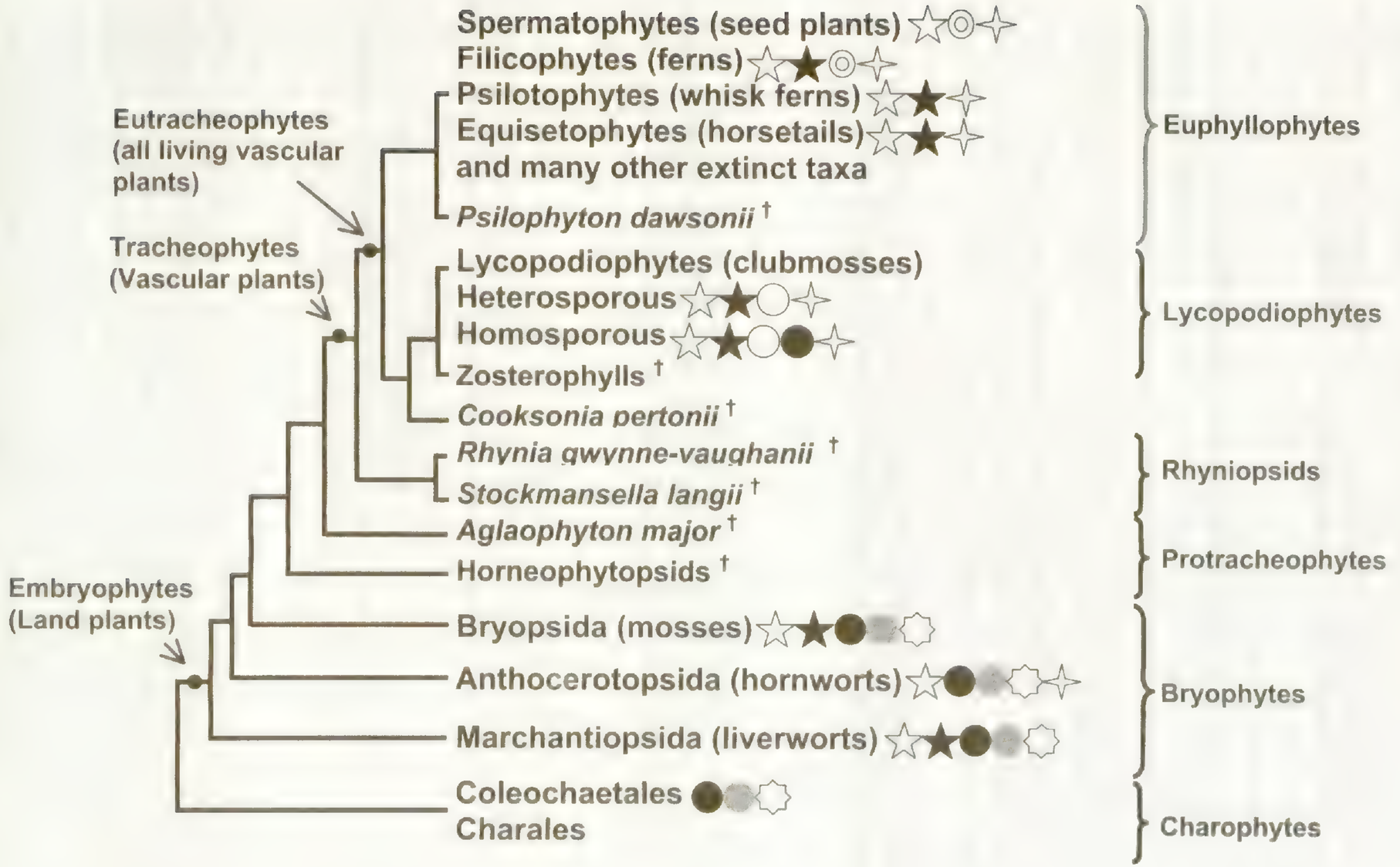


Figure 2. Land plant phylogeny as present by Kenrick and Crane (1987) annotated to show major transitions in cell wall components. The presence of specific cell wall components are shown as follows; xyloglucan,☆; mannose,★; 3-O-methylgalactose,⌒; 3-O-methylrhamnose,●; GalA,◍; Tannin,◎; GlcA,{◌}; branched 4-linked xylan,^ v . Extinct plants, indicated by †, which represent large gaps in plant morphology are included.

psilotophytes, eusporangiate and leptosporangiate ferns proposed by Pryer *et al.* (2001). However, the acquisition of tannins may be homoplasic having evolved at the same time within the spermatophyte and filicophyte groups due to a common selection pressure. Schneider *et al.* (2004) suggest polypod ferns diversified (~ 180 million years ago) subsequent to radiation of the angiosperms (~250 million years ago). Evidence from cell wall characters is therefore seen in general to partially corroborate an hypothesised early mid-Devonian split within the euphyllophytes and subsequent concurrent evolution of seed plants and pteridophytes.

Cell wall enzymes

A complete description of the enzymes involved in plant cell wall biosynthesis and modification has not been obtained and few of the membrane-bound polysaccharide synthases have been biochemically characterised (Burton *et al.*, 2000). I will briefly discuss some enzyme families (Csl, CesA and XTH) whose function has been at least partially elucidated.

Cellulose is one of the most abundant organic molecules on the planet with plants synthesising more than 10^{11} metric tons per year (Hess *et al.*, 1928). Proteins that have a role in cellulose synthesis are encoded by a large family of cellulose synthase genes (CesA). All members of the CesA gene family isolated from land plants encode for integral membrane proteins which share many conserved regions (Richmond & Somerville, 2000; Vergara & Carpita, 2001) and have some molecular motifs identical to those found in CesA proteins from the green alga *Mesotanium caldariorum* (Roberts *et al.*, 2002; Roberts & Roberts, 2004). Genes within the CesA family are thought to be functionally non-redundant owing to the arrangement of the proteins they encode in structurally well-defined transmembrane complexes (Kimura *et al.*, 1999). A gene superfamily known as cellulose synthase-like (Csl) genes has also been described (Richmond & Somerville, 2000). Proteins encoded by these genes are likely to be involved in the synthesis of hemicellulosic polysaccharides including xyloglucan, xylan and glucomannan (Liepman *et al.*, 2005; Dhugga *et al.*, 2004).

Xyloglucan endotransglycosylases/hydrolases (XTH's) are a class of enzymes that transglycosylate xyloglucan. XTH's are encoded by at least 33 genes in *Arabidopsis* and recent studies have revealed that individual members of this gene family exhibit specific temporal and spatial expression patterns (Matsui *et al.*, 1995). Two loss-of-function *Arabidopsis* mutants of the AtXTH27 gene (XTH27-1 and XTH27-2) have short tracheary elements in the tertiary veins and a reduced number of tertiary veins in the first leaf. The highest level of AtXTH27 mRNA expression was seen during leaf expansion where the tracheary elements were elongating. The AtXTH27 gene therefore appears to have a role in cell wall modification during development of tracheary elements (Matsui *et al.*, 1995). Vissenberg *et al.* (2003) have shown that the primary cell walls of lycopodiophytes, early diverging pteridophytes and the earliest diverging extant vascular plants (Raubeson & Jansen, 1992; Manhart, 1994, 1995; Pryer *et al.*, 1995; Wolf, 1997; Duff & Nickrent, 1999) contain XTH activity. Owing to the control of tracheary element elongation by an XTH and because XTH activity has been detected in sporophyte and gametophyte tissues from the liverwort *Marchantia* and in gametophyte tissue from a moss, *Mnium* (Fry *et al.*, 1992) it is likely that divergence of the XTH genes played a role in the evolution of vascular plants.

The existence of multiple members of Csl, CesA, and XTH gene families allows for temporal and spatial differences in gene expression and may account for differences in

cell wall polysaccharide composition and morphologies among various plant groups and between different tissues within the same plant.

Cell wall-associated proteins

Cell wall-associated proteins may directly influence plant morphogenesis. Morphogenesis in plants is the result of differential growth of the organs at the level of cell walls (Kaplan & Hageman, 1991). The cell wall-associated proteins known as arabinogalactan proteins have a diverse range of structures and functions. In large part, heterogeneity found in arabinogalactan proteins is within the carbohydrate domain (Gaspar *et al.*, 2001). This diversity may allow for subtle differences in function. Much of the evidence relating to arabinogalactan distribution and function has been based on the use of monoclonal antibodies which react with carbohydrate epitopes within arabinogalactan proteins (McCabe *et al.*, 1997; Casero *et al.*, 1998). Arabinogalactan proteins are thought to play a major role in cell–cell interactions and have been shown to have a morpho-regulatory role in bryophyte (Basile, 1980; Basile & Basile, 1983, 1987) and higher plant (Kreuger & van Holst, 1996; Majewska-Sawka & Nothnagel, 2000; Johnson *et al.*, 2003) development. Arabinogalactan proteins are synthesised by the moss *Physcomitrella patens*, where they appear to have a role in regulating the extension of protonemal cells with apical tip growth (Lee *et al.*, 2005). Thus, arabinogalactan proteins, and in particular their carbohydrate domain, may be important agents defining different body-plans of all land plants and thus pivotal in the evolution of the major groups of land plants.

Expansins are wall proteins encoded by a multigene family. These proteins modify the mechanical properties of cell walls, allowing turgor-driven cell enlargement (Cosgrove, 2000). There are two subfamilies of expansins, α and β. The α-expansins have a highly conserved protein sequence and are found in all embryophyte taxa (land plant groups) including the aquatic ferns, *Marsilea* and *Regnellidium* (Kim *et al.*, 2000) and the moss *Physcomitrella patens* (Li *et al.*, 2002) whereas β-expansins are present in low concentration in dicotyledonous angiosperms and occur at a much higher concentration in members of the monocotyledonous Poales (Cosgrove, 2000). Expansins are believed have a prominent role in xylem development (Cosgrove, 2000) and thus their evolution may be closely associated with that of tracheophytes.

ECOLOGICAL CONSEQUENCES OF CELL WALL DIVERSITY

Variation in cell wall composition influences which pathogens can infect a plant. Cell walls are a physical barrier to bacterial, viral and fungal pathogens. However, many pathogens secrete enzymes that degrade cell wall components, those which degrade polysaccharides being among the most specific. Most glycanases can only cleave the glycosidic linkage between two specific monosaccharides and often that bond must be between two particular carbon atoms and of a specific anomeric configuration. For example, endopolygalacturonases can only cleave the glycosidic bond between 1, 4-linked α-D-galacturonic acid residues. Thus, changing the type of glycosidic bond may alter the susceptibility of a polysaccharide to enzyme hydrolysis (Albersheim *et al.*, 1969). Cell wall composition is therefore likely to be a factor that determines which fungi and bacteria are able to be pathogenic to particular plants.

Decomposers synthesise specific cell wall-degrading enzymes. Not all saprophytes will be able to synthesise every enzyme required to degrade all the polysaccharides in a cell wall. Thus, saprophytes tend to work synergistically to degrade plant material.

Differences in cell wall biochemistry between a fern-rich as opposed to an angiosperm or gymnosperm-rich community may be reflected by differences in the decomposers acting on the plant materials present. Therefore, cell wall biochemistry may be an important factor determining fungal and bacterial biodiversity.

CONCLUSIONS

The transition from an aqueous to an initially low-competition gaseous medium exposed plants to new physical conditions and resulted in selection for key physiological and structural changes. Early plant terrestrialisation is predicted to have rapidly filled all niches where water availability was limited (Bateman *et al.*, 1998), thereby increasing competition and driving selection for turgor-stabilised upright stems and decreased dependence on water availability. Mutations that enabled plants to adopt a more upright growth habit, many of which are likely to have been cell wall related, would have been strongly favoured. It is likely that these evolutionary pressures brought about the differences in cell wall composition that occurred during the colonisation of land and during the emergence of the vascular and leptosporangiate conditions. Major differences in cell wall composition between different plant taxa are summarised in figure 2. Differences appear to provide specific traits that appear to be landmarks. Specifically, the presence of the primary cell wall polysaccharide, xyloglucan, in all land plants, but apparent absence from their closest extant common ancestors, the charophycean green algae (Popper & Fry, 2003, 2004), implies that xyloglucan may have been required for land colonisation or that the subsequent evolution of land plants required walls that contained xyloglucan. Attainment of the vascular condition is associated in secondary cell walls with a reduction in total primary cell wall uronic acid content (Popper & Fry, 2003, 2004) and the presence of highly substituted xylans (Carafa *et al.*, 2005). Complex xylans also occur in hornwort cell walls which, contrary to the phylogeny presented in figure 2 (Kenrick & Crane, 1997), may suggest that tracheophytes are more closely related to hornworts than they are to liverworts and mosses. Vascular plants are unified by the presence of rhamnogalacturonan II in their primary cell walls (Matsunaga *et al.*, 2004). Rhamnogalacturonan II and the acquisition of a boron-dependant growth habit together with branched xylans are likely to have been important in evolution of upright stem tissues. Among vascular plants, there is a pronounced chemical demarcation between eusporangiate pteridophytes (high mannan, low tannin) and leptosporangiate ferns (low mannan, high tannin) (Popper & Fry, 2004). The emergence and divergence of the pteridophytes appears, therefore, to be associated with a period of modification in cell wall composition.

Stebbins (1992) has stated that 'even the most superficial survey of ecological plant anatomy points to the adaptive importance of cell wall differentiation'. Indeed, the present literature shows changes in cell wall polysaccharide composition and expression of cell wall proteins and enzymes are likely to have been fundamental to the emergence, divergence and survival of extant ferns.

ACKNOWLEDGMENTS

Z.A.P. thanks Glenn Freshour for Figure 1 depicting monoclonal antibody labelling of *Equisetum* cell walls, and Malcolm O'Neill and Michael Hahn for useful discussions and critical reading of this manuscript.

REFERENCES

AKIYAMA, T., TANAKA, K., YAMAMOTO, S. & ISEKI S. 1988. Blood-group active proteoglycan containing 3-*O*-methylrhamnose (acofriose) from young plants of *Osmunda japonica*. Carbohydr. Res. 178: 320–326.

ALBERSHEIM, P., JONES, T.M. & ENGLISH, P.D. 1969. Biochemistry of the plant cell wall in relation to infective processes. Ann. Rev. Phytopath. 7: 171–194.

ALBERSHEIM, P. 1976. Plant Biochemistry. Bonner, J, Varner, JE, eds. New York, NY, USA: Academic Press

ANDERSON, D.M.W. & MUNRO, A.C. 1969. The presence of 3-*O*-methylrhamnose in *Araucaria resinous* exudates. Phytochemistry 8: 633–634.

BASILE, D.V. 1980. A possible mode of action for morphoregulatory hydroxproline-proteins. Bull. Torrey Bot. Club 107: 325–338.

BASILE, D.V. & BASILE, M.R. 1983. The role and control of the place-dependant suppression of cell division in plant morphogenesis and phylogeny. Mem. Torrey Bot. Club 25: 63–84.

BASILE, D.V. & BASILE, M.R. 1987. The occurrence of cell wall-associated arabinogalactan proteins in the Hepaticae. Bryologist 90: 401–404.

BATEMAN, R.M., CRANE, P.R., DIMICHELE, W.A., KENRICK, P.R., ROWE, N.P., SPECK, T. & STEIN, W.E. 1998. Early evolution of land plants: phylogeny, physiology and ecology of the primary terrestrial radiation. Ann. Rev. Ecol. Syst. 29: 263–292.

BATE-SMITH, E.C. & LEARNER, N.H. 1954. Leuco-anthocyanins. 2. Systematic distribution of the leuco-anthocyanins in leaves. Biochem. J. 58: 126–132.

BOYCE, C.K., CODY, G.D., FOGEL, M.L., HAZEN, R.M., ALEXANDER, C.M. O'D. & KNOLL, A.H. 2003. Chemical evidence for cell wall lignification and the evolution of tracheids in early Devonian plants. Intl. J. Pl. Sci. 164: 691–702.

BRETT, C.T. & WALDRON, K.W. 1996. Physiology and biochemistry of plant cell walls. London: Chapman and Hall.

BREMNER, I. & WILKIE, K.C.B. 1971. The hemicelluloses of bracken. II. A galactoglucomannan. Carbohyd. Res. 20: 193–203.

BROWN, R.C. & LEMMON, B.E. 1993. Diversity of cell division in simple land plants holds clues to evolution of the mitotic and cytokinetic apparatus in higher plants. Mem. Torrey Bot. Club 25: 45–62.

BURKE, D., KAUFMAN, P., MCNEIL, M. & ALBERSHEIM, P. 1974. The structure of plant cell walls. VI. A survey of the walls of suspension-cultured monocots. Pl. Physiol. 54: 109–115.

BURTON, R.A., GIBEAUT, D.M., BACIC, A., FINDLAY, K., ROBERTS, K., HAMILTON, A., BAULCOMBE, D.C. & FINCHER, G.B. 2000. Virus-induced silencing of a plant cellulose synthase gene. Plant Cell 12: 691–705.

CARAFA, A., DUCKETT, J.G., KNOX, J.P. & LIGRONE, R. 2005. Distribution of cell wall xylans in bryophytes and tracheophytes: new insights into basal interrelationships of land plants. New Phytol. 168: 231–240.

CARPITA, N. 1996. Structure and biogenesis of cell walls of grasses. Ann. Rev. Pl. Physiol. Pl. Mol. Biol. 47: 445–476.

CARPITA, N.C. & GIBEAUT, D.M. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant Journal 3: 1–30.

CASERO, P.J., CASIMIRO, I. & KNOX, J.P. 1998. Occurrence of cell surface

arabinogalactan-protein and extensin epitopes in relation to pericycle and vascular tissue development in the root apex of four species. Planta 204: 252–259.

CASSAB, G.I. 1998. Plant cell wall proteins. Ann. Rev. Pl. Physiol. Pl. Mol. Biol. 49: 281–309.

COSGROVE, D.J. 2000. New genes and new biological roles for expansins. Curr. Opinion Pl. Biol. 3: 73–78.

CÔTÉ, F. & HAHN, M.G. 1994. Oligosaccharins : Structure and signal transduction. Pl. Mol. Biol. 26 : 1379–1411.

CÔTÉ, F., HAM, K-S., HAHN, M.G. & BERGMAN, C.W. 1998. Oliogosaccharide elicitors in host-pathogen interactions : generation, perception, and signal transduction In : Biswas, B.B. & Das, H. (eds) Cellular Biochemistry — Plant-microbe interactions Volume 29 pp. 385–432. New York, Plenum publishing corporation.

DE BRUYNE, T., PIETERS, L., DEELSTRA, H. & VLIETINCK, A. 1999. Condensed vegetable tannins: Biodiversity in structure and biological activities. Biochem. Syst. Ecol. 27: 445–459.

DHUGGA, K.S., BARREIRO, R., WHITTEN, B., STECCA, K., HAZENBROEK, J., RANDHAWA, G.S., DOLAN, M., KINNEY, A.J., TOMES, D., NICHOLS, S. & ANDERSON, P. 2004. Guar seed β-mannan synthase is a member of the cellulase synthase supergene family. Science 303: 363–366.

DUFF, J.R. & NICKRENT, D.L. 1999. Phylogenetic relationships among land plants using mitochondrial small-subunit rDNA sequences. Am. J. Bot. 86: 372–386.

DUMVILLE, J.C. & FRY, S.C. 1999. Uronic acid-containing oligosaccharins: their biosynthesis, degradation and signalling roles in non-diseased plant tissues. Pl. Physiol. Biochem. 38: 125–140.

EDA, S., AKIYAMA, Y. & KATO, K. 1985. A galactoglucomannan from cell walls of suspension-cultured tobacco (*Nicotiana tabacum*) cells. Carbohyd. Res. 137: 173–181.

EDASHIGE, Y. & ISHII, T. 1996. Structures of cell-wall polysaccharides from suspension-cultured cells of *Cryptomeria japonica*. Mokuzai Gakkaishi 42: 895–900.

FOWLER, J.E. & QUATRANO, R.S. 1997. Plant cell morphogenesis: Plasma membrane interactions with the cytoskeleton and cell wall. Ann. Rev. Cell Dev. Biol. 13: 697–743.

FRESHOUR, G., CLAY, R.P., FULLER, M.S., ALBERSHEIM, P., DARVILL, A.G. & HAHN, M.G. 1996. Developmental and tissue-specific structural alterations of the cell wall polysaccharides of *Arabidopsis thaliana* roots. Pl. Physiol. 110: 1413–1429.

FRY, S.C. 1999. Plant cell walls. In: Encyclopedia of life sciences. London: Nature Publishing Group. http://www.els.net

FRY, S.C. 2000. The growing plant cell wall: chemical and metabolic analysis, reprint edition. Caldwell NJ, USA: The Blackburn Press.

FRY, S.C, SMITH, R.C., RENWICK, K.F., MARTIN, D.J., HODGE, S.K. & MATTHEWS, K.J. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem. J. 282: 821–828.

GASPAR, Y., JOHNSON, K.L., McKENNA, J.A., BACIC, A. & SCHULTZ, C.J. 2001. The complex structures of arabinogalactan-proteins and the journey towards understanding function. Pl. Mol. Biol. 47: 161–176.

GEDDES, D.S. & WILKIE, K.C.B. 1971. Hemicelluloses from the stem tissues of the aquatic moss *Fontinalis antipyretica*. Carbohyd. Res. 18: 333–335.

GEDDES, D.S. & WILKIE, K.C.B. 1972. A galactoglucomannan from the stem tissues of the aquatic moss *Fontinalis antipyretica*. Carbohyd. Res. 23: 349–357.

GERHART, J. & KIRSHNER, M. 1997. Cells, embryos and evolution. Toward a cellular and developmental understanding of phenotypic variation and evolutionary adaptability. Malden, MA, USA: Blackwell Science Inc.

GIDDINGS, T.H., BROWER, D.L. & STAEHELIN, L.A. 1980. Visualization of particle complexes in the plasma membrane of *Micrasterias denticulata* associated with the formation of cellulose fibrils in primary and secondary cell walls. J. Cell Biol. 84: 327–339.

GOLDBERG, R., PRAT, R. & MORVAN, C. 1994. Structural features of water soluble pectins from mung bean hypocotyls. Carbohyd. Polymers 23: 203–210.

GOTTELI, L.B. & CLELAND, R. 1968. Differences in the occurrence and distribution of hydroxyproline-proteins among the algae. Am. J. Bot. 55: 907–914.

HESS, K., HALLER, R. & KATZ, J.R. 1928. Die Chemie der Zellulose und ihrer Begleiter: Leipzig (Germany) Akademische Verlagsgesellschaft.

JOHNSON, K.L., JONES, B.J., BACIC, A. & SHULTZ, C.J. 2003. The fasiclin-like arabinogalactan proteins of *Arabidopsis*. A multigene family of putative cell adhesion molecules. Pl. Physiol. 133: 1911–1925.

JONES, L., SEYMOUR, G.B. & KNOX, J.P. 1997. Localisation of pectic galactan in tomato cell walls using a monoclonal antibody specific to (1→4)-β-D-galactan. Pl. Physiol. 113: 1405–1412.

JUDD, W.S., CAMPBELL, C.S., KELLOGG, E.A. & STEVENS, P.F. 1999. Plant Systematics a Phylogenetic Approach. Sunderland, MA, USA: Sinauer associates Inc.

KAPLAN, D.R. & HAGEMAN, W. 1991. The relationship of cell and organism in vascular plants. Bioscience 41: 693–703.

KENRICK, P. & CRANE, P.R. 1997. The origin and early evolution of plants on land. Nature 389: 33–39.

KIM, J-H., CHO, H.T. & KENDE, H. 2000. α-Expansins in the semiaquatic ferns *Marsilea quadrifolia* and *Regnellidium diphyllum*: evolutionary aspects and physiological role in rachis elongation. Planta 212: 85–92.

KIMURA, S., LAOSINCHAI, W., ITOH, T., CUI, X., LINDER, C.R. & BROWN, R.M. 1999. Immunogold labelling of rosette terminal cellulose-synthesising complexes in the vascular plant *Vigna angularis* Plant Cell 11: 2075–2086.

KNOX, J.P. 1997. The use antibodies to study the architecture and developmental regulation of plant cell walls. Intl. Rev. Cytol. 171: 79–120.

KREMER, C., PETTOLINO, F., BACIC, A., DRINNAN, A. 2004. Distribution of cell wall components in *Sphagnum* hyaline cells and in liverwort elators. Planta 219: 1023–1035.

KREUGER, M. & VAN HOLST, G.J. 1996. Arabinogalactan proteins and plant differentiation. Pl. Mol. Biol. 30: 1077–1086.

LEE, K.J.D., SAKATA, Y., MAU, S-L., PETTOLINO, F., BACIC, A., QUATRANO, R.S., KNIGHT, C.D. & KNOX, J.P. 2005. Arabinogalactan proteins are required for apical cell extension in the moss Physcomitrella patens. Plant Cell 17: 3051–3065.

LI, Y., DARLEY, C.P., ONGARO, V., FLEMING, A., BALDAUF, O.S. & McQUEEN-MASON S.J. 2002. Plant expansins are a complex multigene family

with an evolutionary origin. Pl. Physiol. 128: 854–864.
LIEPMAN, A.H., WILKERSON, C.G. & KEEGSTRA, K. 2005. Expression of cellulose synthase-like (Csl) genes in insect cells reveals that CslA family members encode mannan synthases. Proc. Natl. Acad. Sci., U.S.A. 102: 2221–2226.
MAJEWSKA-SAWKA, A. & NOTHNAGEL, E.A. 2000. The multiple roles of arabinogalactan proteins in plant development. Pl. Physiol. 122: 3–9.
MANHART, J.R. 1994. Phylogenetic analysis of green plant rbcL sequences. Molecular Phylogenetics and Evolution 3: 114–127.
MANHART, J.R. 1995. Chloroplast 16S rRNA sequences and phylogenetic relationships of fern allies and ferns. Am. Fern J. 85: 182–192.
MARCHANT, H.J. & PICKETT-HEAPS, J.D. 1973. Mitosis and cytokinesis in *Coleochaete scutata*. J. Phycol. 9: 461–471.
MATSUI, A., YOKOYAMA, R., SEKI, M., SHINOZAKI, K., TAKAHASHI, T., KOMEDA, Y. & NISHITANI, K. 1995. AtXTH27 plays an essential role in cell wall modification during development of tracheary elements. Plant J. 42: 525–534.
MATSUNAGA, T., ISHII, T., MATSUMOTO, S., HIGUCHI, M., DARVILL, A.G., ALBERSHEIM, P. & O'NEILL, M.A. 2004. Occurrence of the primary cell wall polysaccharide rhamnogalacturonan II in pteridophytes, lycophytes and bryophytes. Implications for the evolution of vascular plants. Pl. Physiol. 134: 339–351.
McCABE, P.F., VALENTINE, T.A., FORSBERG, L.S. & PENNELL, R.I. 1997. Soluble signals from cells identified at the cell wall establish a developmental pathway in carrot. Plant Cell 9: 2225–2241.
McCARTNEY, L., MARCUS, S.E. & KNOX, J.P. 2005. Monoclonal antibodies to plant cell wall xylans and arabinoxylans. J. Hist. Cytochem. 53: 543–546.
McCARTNEY, L., GILBERT, H.J., BOLAM, D.W., BORASTON, A.B. & KNOX, J.P. 2004. Glycoside hydrolase carbohydrate binding modules as molecular probes for the analysis of plant cell wall polymers. Analytical Biochem. 326: 49–54.
McNEIL, M., DARVILL, A.G., FRY, S.C. & ALBERSHEIM, P. 1984. Structure and function of primary cell walls of plants. Ann. Rev. Biochem. 53: 625–663.
MISHIMA, T., HISAMATSU, M., YORK, W.S., TENANISHI, K. & YAMADA, T. 1998. Adhesion of β-D-glucans to cellulose. Carbohyd. Res. 308: 389–395.
MURATA, T. & WADA, M. 1989. Organization of cortical microtubules and microfibril deposition in response to blue-light-induced apical swelling in a tip-growing *Adiantum* protonema cell. Planta 178: 334–341.
NEWMAN, R.H. 1997. Crystalline forms of cellulose in the silver tree fern *Cyathea dealbata*. Cellulose 4: 269–279.
NIKLAS, K.J. 2005. The cell walls that bind the tree of life. BioScience 54: 831–841.
O'NEILL, M.A., EBERHARD, S., ALBERSHEIM, P. & DARVILL, A.G. 2001. Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth. Science 294: 846–849.
O'NEILL, M.A., ISHII, T., ALBERSHEIM, P. & DARVILL, A.G. 2003. Rhamnogalacturonan II: Structure and function of a borate cross-linked cell wall pectic polysaccharide. Ann. Rev. Pl. Biol. 55: 109–139.
PICKETT-HEAPS, J.D. & NORTHCOTE, D.H. 1966. Organisation of microtubules and endoplasmic reticulum during mitosis and cytokinesis in wheat meristems. J. Cell Sci. 1: 109–120.
POPPER, Z.A., SADLER, I.H. & FRY, S.C. 2001. 3-*O*-Methyl-D-galactose residues in lycophyte primary cell walls. Phytochem. 57: 711–719.

POPPER, Z.A. & FRY, S.C. 2003. Primary cell wall composition of bryophytes and charophytes. Ann. Bot. 91: 1–12.

POPPER, Z.A. & FRY, S.C. 2004. Primary cell wall composition of pteridophytes and spermatophytes. New Phytol. 164: 165–174.

PRYER, K.M., SMITH, A.R. & SKOG, J.E. 1995. Phylogenetic relationships of extant ferns based on evidence from morphological and rbcL sequences. Am. Fern J. 85: 205–282.

PRYER, K.M., SCHNEIDER, H., SMITH, H.R., CRANFILL, R., WOLF, P.G., HUNT, J.S. & SIPES, S.D. 2001. Horsetails and ferns are a monophyletic group and are the closest living relatives to seed plants. Nature 409: 618–622.

PUHLMANN, J., BUCHELI, E., SWAIN, M.J., DUNNING, N., ALBERSHEIM, P., DARVILL, A.G. & HAHN, M.G. 1994. Generation of monoclonal antibodies against plant cell wall polysaccharides. I. Characterisation of a monoclonal antibody to a terminal α-(1,2)-linked fucosyl-containing epitope. Pl. Physiol. 104: 699–710.

RACUSEN, R.H. 2002. Early development in fern gametophytes : interpreting the transition to prothalli architecture in terms of coordinated photosynthate production and osmotic ion uptake. Ann. Bot. 89: 227–240.

RAUBESON, L.A. & JANSEN, R.K. 1992. Chloroplast DNA evidence on the ancient evolutionary split in vascular land plants. Science 255: 1697–1699.

RAVEN, J.A. 1993. The evolution of vascular plants in relation to quantitative functioning of dead water-conducting cells and stomata. Biol. Rev. 68: 337–363.

RICHMOND, T.A. & SOMERVILLE, C.R. 2000. The cellulose synthase superfamily. Pl. Physiol. 124: 495–498.

ROBERTS, A.W. & ROBERTS, E.M. 2004. Cellulose synthase (CesA) genes in the green algae and seed plants. Cellulose 11: 419–435.

ROBERTS, A.W., ROBERTS, E.M. & DELMER, D.P. 2002. Cellulose synthase (CesA) genes in the green alga *Mesotaenium caldariorum*. Eukaryotic Cell 1: 847–855.

RYDEN, P., SUGIMOTO-SHIRASU, K., SMITH, A.C., FINDLAY, K., REITER, W.D. & MCCANN, M.C. 2003. Tensile properties of *Arabidopsis* cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II-borate complexes. Pl. Physiol. 132: 1033–1040.

SAXENA, I.M. & BROWN, R.M. 2005. Cellulose biosynthesis: current views and evolving concepts. Ann. Bot. 96: 9–21.

SCHNEIDER, H., SCHUTTPELZ, E., PRYER, K.M., CRANFILL, R., MAGALLÓN, S. & LUPIA, R. 2004. Ferns diversified in the shadow of angiosperms. Nature 428: 553–557.

SHATALOV, A.A., EVTUGUIN, D.V. & NETO, C.P. 1999. (2-*O*-α-D-galacturonosyl-4-*O*-methyl-α-D-glucurono)-D-xylan from *Eucalyptus globus* Labill. Carbohyd. Res. 320: 93–99.

SMITH, A.R. 1995. Non-molecular phylogenetic hypotheses for ferns. Am. Fern J. 85: 104–122.

SMITH, B.G. & HARRIS, P.J. 1999. The polysaccharide composition of Poales cell wall: Poaceae cell walls are not unique. Biochem. Syst. Ecol. 27: 33–53.

STEBBINS, G.L. 1992. Comparative aspects of plant morphogenesis: a cellular, molecular and evolutionary approach. Am. J. Bot. 79: 589–598.

THOMAS, J.R., McNEIL, M., DARVILL, A.G. & ALBERSHEIM, P. 1987. Structure of plant cell walls. 29. Isolation and characterization of wall polysaccharides from

suspension-cultured Douglas Fir cells. Pl. Physiol. 83: 659–671.

TIMELL, T.E. 1962. Studies on ferns (Filicinceae). Part 1: The constitution of a xylan from Cinnamon fern (*Osmunda cinnamomea* L.) Svensk Papperstidning 65: 122–125.

VERGARA, C.E. & CARPITA, N.C. 2001. β-D-glycan synthases and the CesA gene family : lessons to be learned from the mixed-linkage (1–3),(1–4) β-D-glucan synthase. Pl. Mol. Biol. 47: 145–160.

VISSENBERG, K., VAN SANDT, V., FRY, S.C. & VERBELEN, J-P. 2003. Xyloglucan endotransglucosylase action is high in the root elongation zone and in the trichoblasts of all vascular plants from *Selaginella* to *Zea mays*. J. Exp. Bot. 54: 335–346.

WADA, M. & STAEHELIN, L.A. 1981. Freeze-fracture observations on the plasma membrane, the cell wall and the cuticle of growing protonemata of *Adiantum capillus-veneris* L. Planta 151: 462–468.

WILLATS, W.G.T., MARCUS, S.E. & KNOX, J.P. 1998. Generation of a monoclonal antibody specific to (1→5)-α-L-arabinan. Carbohyd. Res. 308: 149–152.

WILLATS, W.G.T., STEELE-KING, C.G., MCCARTNEY, L., ORFILA, C., MARCUS, S.E. & KNOX, J.P. 2000. Making and using antibody probes to study plant cell walls. Pl. Physiol. Biochem. , 38, 27–36

WOLF, P.G. 1997. Evaluation of *atpB* nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. Am. J. Bot. 84: 1429–1440.

TRICHOMANES SPECIOSUM (HYMENOPHYLLACEAE: PTERIDOPHYTA) IN NORTHWESTERN FRANCE

S. LORIOT[1, 2], S MAGNANON[1] & E. DESLANDES[2]

[1] Conservatoire Botanique National de Brest. 52, allée du Bot 29200 Brest France (Email: sandrine.loriot@univ-brest.fr)

[2] Laboratoire d'Écophysiologie et de Biotechnologie des Halophytes et des Algues Marines. Institut Universitaire Européen de la Mer. Université de Bretagne Occidentale. Technopôle Brest-Iroise 29280 Plouzané France

Key words: *Trichomanes speciosum* Willd., sporophyte, gametophyte, habitat, distribution, abundance, ecology, north-western France

ABSTRACT

With the aim to establish a conservation plan for the endangered *Trichomanes speciosum* Willd. in northwestern France, the "Conservatoire Botanique National" of Brest has carried out field work to collect appropriate information about this species. In particular, the data available about the type of habitat, abundance and ecology ,as well as the threats for the gametophyte and sporophyte stages, were updated.

INTRODUCTION

The Killarney fern, *Trichomanes speciosum* Willd. (*Hymenophyllaceae*), is one of the rarest and most endangered of the European Pteridophytes (Stace, 1997; Boudrie *in* Olivier *et al.*, 1995). Indeed, despite quite a large Atlantic/Macaronesian distribution area (Ratcliffe *et al.*, 1993; Rich *et al.*, 1995; Boudrie *in* Olivier *et al.*, 1995; Krukowski *et al.*, 2002), populations are scarce and fragmented (Fig. 1). Though a larger distribution of this plant has sometimes been reported, reports of this species from the tropics correspond to closely related, but specifically distinct, taxa (Prelli, 2002). In the British Red Data Book, its status is "Vulnerable" in Europe (Ratcliffe *et al.*, in Wigginton, 1999). The fern is listed in Annexes II and IV of the European Community Habitats and Species Directive (European Community, 1992) and is also mentioned in Annex I of the Bern Convention. According to the IUCN citation, the taxon is considered as "rare" in the World and more precisely "endangered" in France where it is legally protected (Boudrie *in* Olivier *et al.*, 1995). Indeed, the distribution of *T. speciosum* across France illustrates the geographical hiatus existing between known sites of the species with distances exceeding a thousand kilometres. French sites of Killarney Fern have been recorded in three major areas (Figure 1): the Basque Country close to Spain (Jovet, 1933), the Massif Armoricain in north-western France (Louis-Arsène, 1953; des Abbayes *et al.*, 1971; Prelli *et al.*, 1992; Bioret *et al.*, 1994) and the Vosges Mountains close to Luxembourg and Germany (Jérôme *et al.*, 1994; Bizot, 2000a). Between these three poles, recent investigations have discovered other sites in the Ardennes, south of Belgium (Bizot, 2000b), Limousin (Boudrie, 2001) and Tarn (Bizot, 2004), at the northwest and south of the Massif Central, respectively.

It is worth noting that in Central Europe, from Belgium to Poland, *Trichomanes speciosum* is observed only as independent gametophytes (Farrar, 1967; Farrar, 1985). In France, sporophytes have been found only in the Basque Country and Massif Armoricain. The scarcity of observable fronds constitutes one of the peculiarities of the

Killarney Fern: this results from the ability of the prothallial stage to persist by vegetative reproduction without transition to a sporophytic generation as in the classical life-cycle of Pteridophytes. To date, no explanation has been provided for this phenomenon, first pointed out in France in the Massif Armoricain by British botanists on a tour across Huelgoat forest (Prelli *et al.*, 1992); since their discovery, many new sites with independent gametophytes have been identified throughout France. For example, in the Vosges Mountains (Jérôme *et al.*, 1994), a systematic prospecting that started in 1992 increased the number of gametophyte sites to 750 (Jérôme *et al.*, 2001); no sporophytic individuals have been found in any of those sites except for one, where four tiny fronds, firstly described by Rumsey *et al.* (1996) in the British Isles, were present between 1993 and 1997 (Rasbach *et al.*, 1999).

Figure 1. *Trichomanes speciosum* distribution with all records mapped on the 50 km UTM grid system, with ● sporophyte sites, ○ gametophyte sites. Focus on the three main regions where the species is recorded in France: the Basque Country (BC), the Vosges Mountains (VM) and the Massif Armoricain (MA).

Moreover, in the Massif Armoricain, *Trichomanes speciosum* showed another feature strictly related to this region: the populations of sporophytes were all recorded in old wells, but never in natural habitats. In the neighbourhood of the first site discovered in 1949 (Louis-Arsène, 1953), systematic exploration conducted in the 1950s evidenced 178 wells sheltering fronds of the Killarney Fern. One should note that, across this geographical area, the first fronds of the species to be found in two natural sites were observed only as recently as 2002 (Poux *et al.*, 2003). In comparison, in 1960 the Basque Country sheltered 21 sporophyte sites (Boudrie *in* Olivier *et al.*, 1995) located on the shady slopes of ravines in the close vicinity of permanent streams, in agreement with the classical description of the species habitat throughout Europe (Allorge *et al.*, 1941, Ratcliffe *et al.*,1993, Page, 1997). In the British Isles, no Killarney Fern has been recorded in man-made habitats except one gametophyte population in an adit (Rumsey *et al.*, 1998).

In France, recent decades have seen a very rapid decrease of the overall population of *Trichomanes speciosum*, despite the discovery of new sites. Indeed, between 1960 and 1992 the number of sites across the Basque Country was reduced to nearly half, falling from 21 to only 12 sites (Boudrie *in* Olivier *et al.*, 1995). In the Massif Armoricain only 128 wells (out the 178 ones identified in the1950s) were still observed in 1976 (J. Moisan, personal communication, 2003). Moreover, the most recent survey carried out by Moisan and Tournay in 1995 confirmed the dramatic decline of the species in north-western France. Only 43 wells were still found to shelter the delicate fern (Rivière, 1999); indeed, about two-thirds of the other wells had been destroyed or hermetically closed with sheets of metal. Further to these observations, the Conservatoire Botanique National de Brest, in charge of the conservation of endangered plant species across the Massif Armoricain, felt it necessary and urgent to take appropriate measurements for the protection and conservation of *T. speciosum*. The pre-requisite for the development and implementation of a conservation plan for the fern was the description of the species' parameters, type of habitat, abundance, ecology and possible threats for the gametophyte and sporophyte stages, which were not available at that time. In particular, concerning the independent gametophytes, no data were available about their distribution and abundance due to the very recent awareness of this stage (1991) by French botanists. It was, therefore, of paramount importance to update these data and collect additional information about the ecology of each generation of the species in north-western France. As a consequence, all the sites known to shelter some stage of Killarney Fern across the Massif Armoricain have been visited so as to characterise the species habitats. Particular attention has been paid to the description of the forested habitat because of the apparent lack of sporophytes in the region. Conservation of *T. speciosum* can then proceed through the definition and implementation of a management policy meeting the environmental conditions required for maintenance of the fern.

MATERIAL AND METHODS

Material

The Killarney Fern is a terrestrial species, and more precisely a filicophyte with an anisomorph digenetic cycle (Cusset, 1997). Classically, by alternation of the haplophase and diplophase, the plant adopts two distinct forms (Des Abbayes *et al.*, 1971). The filamentous gametophytes are found as small, light green and loosely-tangled clumps. These clumps can be about 1cm thick and constituted of cylindrical and aligned cells

formed successively; their mean length is 150 and 300 μm, with a diameter of 40 to 55 μm (Makgomol *et al.*, 2001). These filaments bear numerous brown and unicellular rhizoids for anchorage to the substratum and nutrition (Rumsey *et al.*, 1998). They are characterised by a cellular septum typically at right angles to the main elongation axis of the filaments, unlike bryophyte protonema (Prelli, 2002). Another feature of *T. speciosum* gametophytes are the truncated gemmiferous cells; each of them bears a propagule composed of about 1 to 20 cells of 0.2 to 1.0mm in length which are responsible for the vegetative reproduction (Makgomol *et al.*, 2001). After dispersion, a typical brown scar is left by the propagule at the surface of the gemmiferous cell. In most species of *Trichomane*s, the gametophytes must be about three years old to become sexually mature (Stockey, 1940). The sexual reproductive structures, *i.e.* the archegonium and antheridium, the female and male organs, may be carried by the same filament.

The sporophyte of the fern is pale green when juvenile, and dark green at the adult stage (Page, 1997). It consists of bi- or tri-pinnate fronds (Stace, 1997) with a long petiole and a triangular limb typically translucent because it is made of a single cell layer (Boodle, 1900), except for the venation. Its more or less ramified rhizomatous axis is long and thin, with a diameter of about 5mm (Makgomol *et al.*, 2001; Stace, 1997); its subtomentous aspect comes from a cover of russet-blackish scales. It bears numerous thin roots involved in the anchorage to the substratum (Jermy *et al.*, 1991). Ten to 30cm long fronds (Prelli, 2002) are formed along the rhizome at every 0.5 to 4cm (Cusset, 1997) but their length can reach 50cm (Makgomol *et al.*, 2001). The sori are located at the edge of the pinnules, with each of them at the nerve end. They are surrounded by a tubular chlorophyllous and cup-shaped involucre, (Prelli, 2002) overtopped, at maturity, by a sporangia-bearing filament (Page, 1997). Spores of the genus *Trichomanes* are chlorophyllous, green, spherical and possess a perispore (Tryon *et al.*, 1990). After collection, their viability seems very short, six days according to Stockey (1940). They are said to germinate and develop very slowly (Page, 1992).

Methods

The Conservatoire Botanique National de Brest asked its 300-strong botanist network to observe and report on the field localisation of *Trichomanes speciosum* across the Massif Armoricain. Thus, all the man-made and natural habitats known to shelter the Killarney Fern over the region were systematically visited and described. Each site was pinpointed on 1:25,000 maps published by the French National Geographic Institute (IGN). Then, an updated map of the distribution *T. speciosum* across the Massif Armoricain was produced. Gametophytes of the fern were searched for at sites where only sporophytes had been identified. At sites of independent gametophyte, 1cm² clump samples were collected under licence and examined under the binocular microscope, eventually revealing the presence of tiny sporophytes. To assess the population density, fronds were counted, and their length was evaluated. Concerning the gametophyte, five classes of abundance were defined as follows from the form and density of the growth and the extent of occupied surface: i) class 1, the occurrence of one to ten scattered clumps (1cm²); ii) class 2, eleven to fifty scattered clumps (1cm²); iii) class 3, from more than fifty scattered clumps (1cm²) to an almost continuous carpet of about 1m²; iv) class 4, an almost continuous carpet of about 1 to 2m² and v) class 5, an almost continuous carpet larger than 2m². These data were statistically analysed (t-test) with the Statistica version 6 software to compare the gametophyte-occupied surfaces in the

natural and human-made habitats. These calculations allowed us to describe precisely the gametophyte abundance with respect to the type of habitat and obtain valuable information about its ecology. For each site, a field form was completed with all the relevant parameters about the presence, abundance and state of the population, and the type of habitat with the most important ecological criteria and existing or potential threats.

RESULTS

Habitats of *Trichomanes speciosum* Willd. in the Massif Armoricain

Figure 2 presents the geographical distribution of *Trichomanes speciosum* across northwestern France. Table 1 lists the characteristics of the 165 known sites. All these data allowed us to establish clearly that, in this area, the main type of habitat for the Killarney Fern is still old wells as previously observed; indeed, 109 of these sites shelter sporophytes or gametophytes or both (Figure 3). More precisely, in 32 wells out of 34 the fronds were always associated with gametophytes. The last two wells, in a very poor

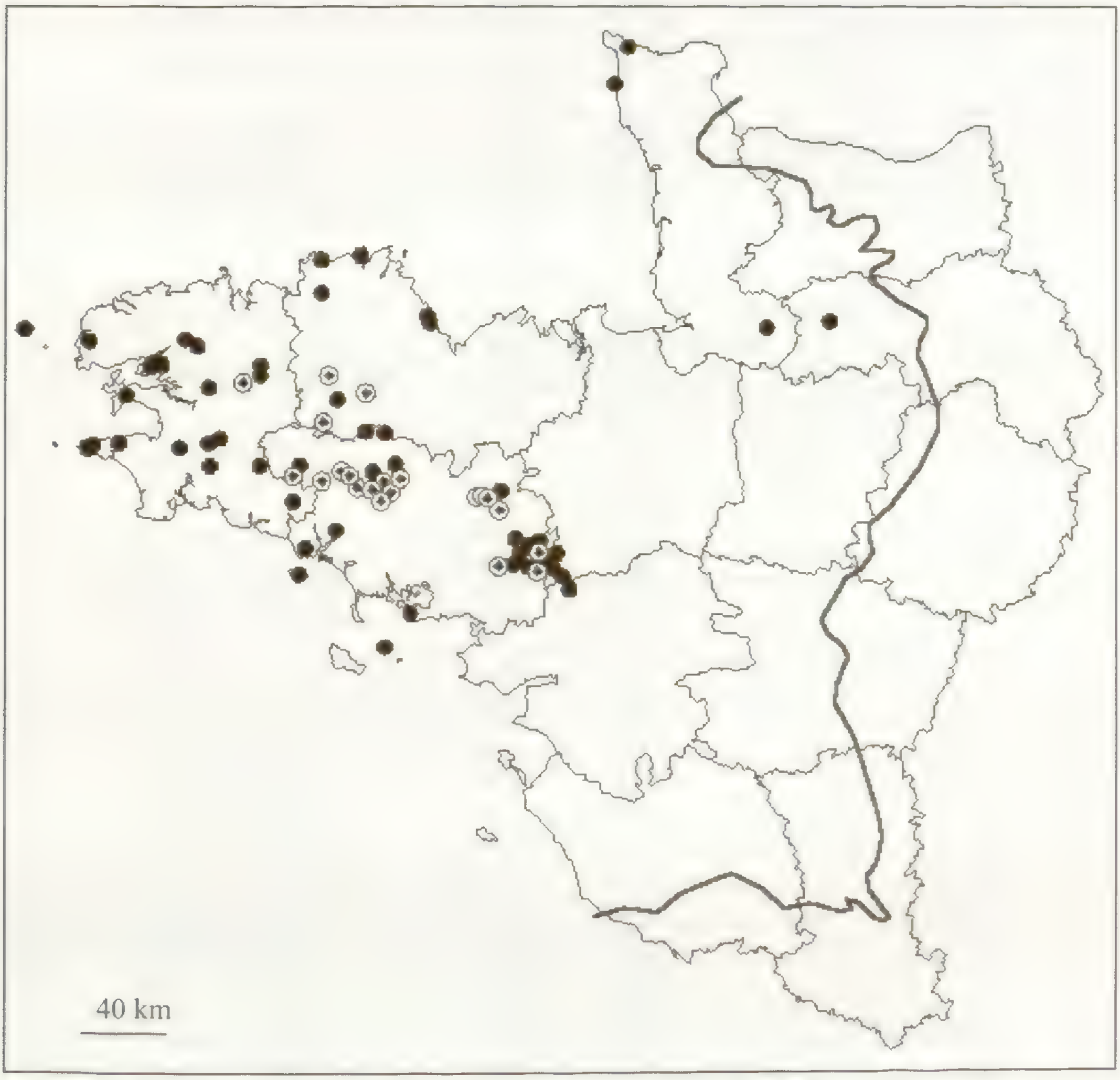


Figure 2. Distribution map of *Trichomanes speciosum* in the Massif Armoricain

◉ commune with a least one population of sporophyte

● commune with at least one independent gametophyte's population but without sporophyte

Table 1. sites of *Trichomanes speciosum* in the Massif Armoricain indicating the Department, the type of habitat and abundance.

Field site	habitat	S	G
Morbihan			
Berne	W	50 *	+
Bieuzy	W	7	+++
	W	0	+++
	W	20 *	+++
Bubry	W	0	+++
	W	0	+
	W	0	+++
	W	15	+++
Carentoir	W	0	+++
Cournon	W	0	+++
	W	0	+++
	W	0	+
La Croix-Helléan	W	50	+++
	W	0	+
	W	0	+
	W	0	+
	W	0	+
	W	0	+++
Faouët	temple	0	++
La Gacilly	W	0	+++
	W	0	+++
	W	0	++
	W	400	+++
Glénac	W	0	+++
	quarry	0	+++++
	W	0	++
Groix	C	0	+++
	C	0	++
Guern	W	0	+
	W	0	+++
	W	0	+++
	W	0	+++
Guiscriff	W	0	inaccessible
Helléan	W	0	++
	W	30 *	+
	W	0	+++
	W	150	++
	W	0	++
Hennebont	cellar	0	++++
Lanvenegen	W	6	+++
	W	20 *	0
	W	50	+++
Lignol	W	80	+++
	W	0	+

Field site	habitat	S	G
Loyat	W	0	++
	W	0	+
Melrand	W	0	+++
	W	0	++
	W	2 △	+++
Peillac	W	0	+
Persquen	W	10	+++
Ploëmeur	C	0	+++
Ploërmel	W	0	+++
	W	0	+
	W	30	+++
	W	19 *	+++
	W	200	+++
	W	0	+++
Pluherlin	W	200	+++
Pluméliau	W	2	+++
Pluméliau	W	20 *	+++
	W	0	++
Pontivy	tunnel	0	+++
Ruffiac	W	0	++
	W	0	+
Saint-Aignan	O	0	+
Saint-Barthélémy.	W	16 *	+++
	W	0	+++
	W	50	+++
Saint-Gildas	C	0	+++
Saint-Gravé	W	0	+
Saint-Martin	W	0	+
	W	0	+
	W	0	+
	W	0	+
	W	0	++
	W	0	+
	W	0	++
Saint-Martin	W	0	+
	W	0	+++
	W	0	++
Saint-Nicolas	W	0	+
	W	0	+++
	W	0	+
Saint-Thuriau	W	15 *	+++
	W	8	+++
Saint-Vincent-sur-Oust	W	100	+++
Taupont	W	10	++
	W	0	+++
	W	200	+++

Field site	habitat	S	G
Taupont	W	6 *	+++
	W	120	+++
	W	0	+
	W	5	++
	W	0	+
	W	0	+
	W	inaccessible *	inaccessible
	W	0	+++
Côtes d'Armor			
Binic	C	0	++
Etables	C	0	++
Glomel	W	100	++
	W	4 *	0
	W	0	++
Kergrist-Moëlou	O	0	++
Perret	O	0	++
Perros-Guirec	C	0	+++
	C	0	+++
Ploubezre	B	0	++
Plougrescant	C	0	++
Saint-Nicolas	B	80 △	+++
Saint-Servais	B	10 △	+++
	B	0	+++
Finistère			
Berrien	O	0	+
Beuzec	C	0	++
Briec	O	0	++
Cléden	C	0	++
	C	0	++
Crozon	C	0	+++
Morgat	C	0	++
Edern	O	0	++
Ergué	O	0	++
Guiclan	O	0	++
Huelgoat	B	0	+
	O	0	+
Locunole	O	0	++
Loqueffret	W	200	+++
Loperec	O	0	++
Loperhet	O	0	+
	O	0	+
Ouessant	C	0	++
	C	0	++
	C	0	+++
	C	0	+++

Field site	habitat	S	G
Plogoff	C	0	++++
	C	0	+++
Plogonnec	O	0	++
Plouarzel	C	0	++
Ploudiry	O	0	++
	O	0	++
Plougastel	O	0	+
	O	0	+
La Roche-Maurice.	O	0	++
Scaër	O	0	++
Ille et Vilaine			
Bains-sur- Oust	W	0	+
	W	0	++
	W	0	+++
	W	0	+++
	W	0	++
	W	0	+
	W	0	+
Sainte-Marie	W	0	+
	W	0	+++
	W	0	+
	W	0	+++
	W	0	+++
	W	0	+++
Sixt-sur-Aff	W	0	+++
	W	0	++
	W	0	++
	W	0	+++
Loire-Atlantique			
Avessac	W	0	++
Manche			
Flamanville	C	0	+++
Gréville	C	0	+++
Mortain	O	0	++
Orne			
Le Châtellier	O	0	+

Type of habitat (W: well; C: coastal cave; O: rocky outcrop, B: woodland boulders; other types of human-made habitat).
Abundance in term of number of fronds for the sporophyte and surface of the overlap for the gametophytes with:
class 1 (+) : 1 to 10 scattered clumps (1cm²)
class 2 (++) : 11 to 50 scattered clumps (1 cm²)
class 3 (+++): from more than 50 scattered clumps to an almost continuous carpet (1 m²)
class 4 (++++) : almost a continuous carpet from 1 to 2 m²
class 5 (+++++) : more than 2 m²
*: dramatically endangered sporophytes whose fronds were observed to be brown and withered
△: small fronds < to 10 cm length

condition, contained no gametophytes, and the fronds were all brown and withered. Conversely, 75 frond-free populations of gametophytes were observed in wells, and four populations were recorded in other types of man-made habitat (Table 1, Figure 3). During this field study, the Killarney Fern was also observed as independent-gametophyte populations in natural habitats, *i.e.* 22 rocky outcrops and five woodland boulders (Table 1, Figure 3). In agreement with a previous report (Poux *et al.*, 2003), two other woodland boulder sites were identified as still sheltering both gametophytes and sporophytes, but their fronds exhibited a quite unusual and intriguing morphology with few denticulations and a very much reduced size. Though well-represented along

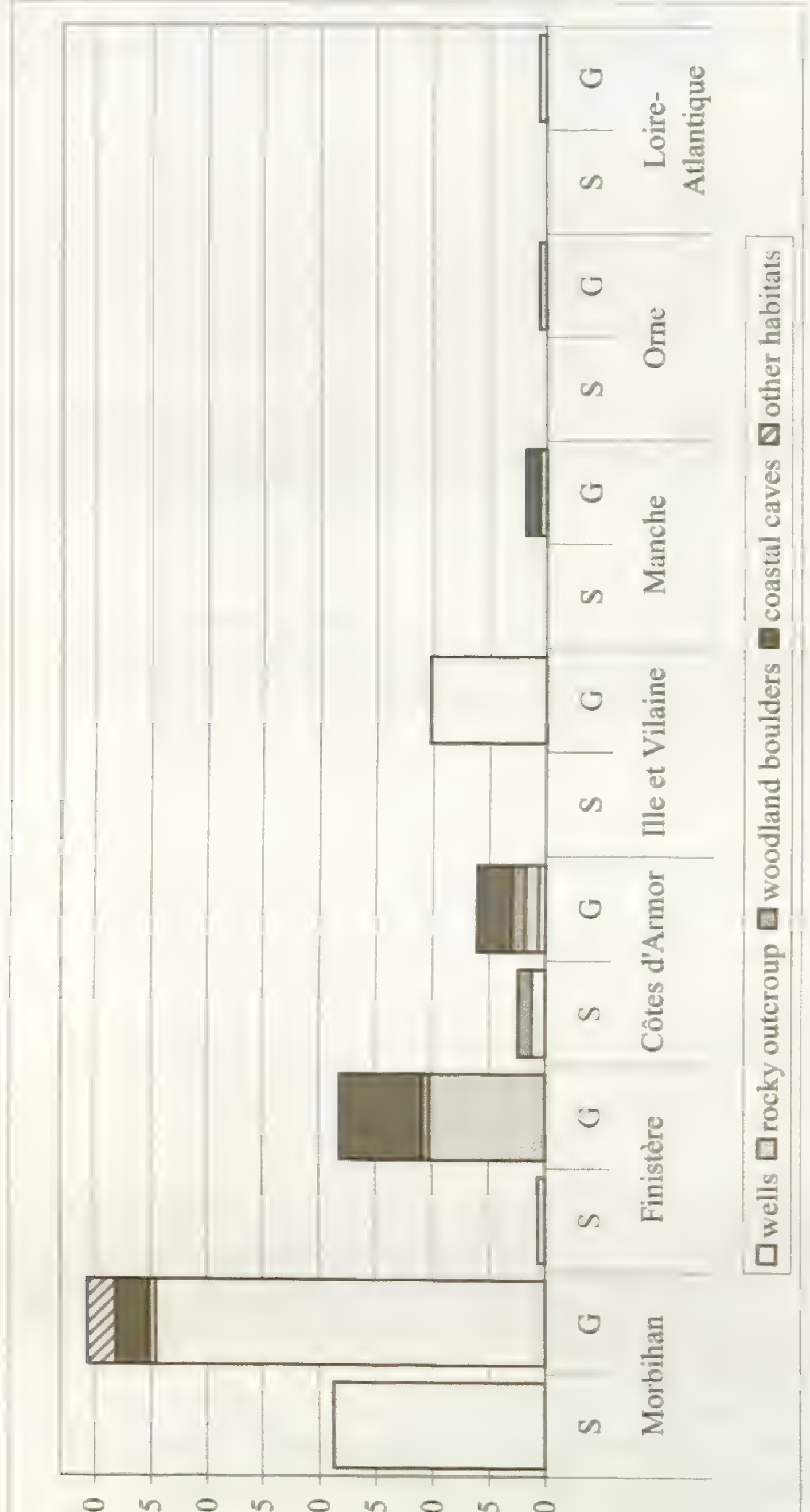


Figure 3. Percentage of sites with sporophytes (S) or independent gametophyte (G) of *Trichomanes speciosum* by department of the Massif Armorican, regarding the type of habitat: well, coastal cave, rocky outcrop, woodland boulders, other types of human-made habitat (quarry, cellar, temple, tunnel).

the shoreline of north-western France, 24 populations were observed in caves, but only as gametophytes (Figure 3).

Abundance of *Trichomanes speciosum*. in the Massif Armoricain

Two to 400 fronds were observed in the wells sheltering sporophytes; their length varied greatly from 8cm (Melrand, *Kerhoh*, Morbihan) to 30cm (La Gacilly, *La Villio*, Morbihan) with a mean size of 15 to 20cm. Some fronds were green and vigorous (Saint-Barthelemy, *Saint-Fiacre*, Morbihan), whereas others were brown and withered (Helléan, *Le Rohello*, Morbihan). A statistical comparison of gametophyte overlap, expressed in percent per class (Figures 4a & 4b), between the natural and man-made habitats revealed very significant differences ($p < 0.01$, $\alpha = 5\%$), but only for classes 2 and 3. Thus, in natural habitats of the Massif Armoricain, the gametophytes were observed mainly as class-2 growths, and in man-made habitats, *e.g.* wells, as class-3 growths.

Ecology of *Trichomanes speciosum* in the Massif Armoricain

Ecological data about each of the 165 sites of the species were collected. Their analysis showed that the natural and human-made habitats of Killarney Fern were always localised on an acid substratum made of sandstone or schistose rocks. *Trichomanes speciosum* was never observed on calcareous formations. In forested habitat, gametophytes were found as independent populations in rocky outcrops and more particularly in small cracks or little flat caverns shielded by a sloping rocky roof. In the woodland boulders, the observed green clumps were either alone or in association with sporophytes on the rockside. As a rule, the Killarney Fern grows on substrates exposed to the North (figure 5); it is never exposed to direct illumination, and it was necessary to use a light source to observe it when present mainly as the gametophyte. In the wells, the prothallial stage always covered the part of the wall below the zone occupied by sporophytes. Such a location corresponds to a cool and wet environment, obviously accentuated by the presence of water in wells or streams in natural habitats. In the natural site of Saint-Nicolas-du-Pelem (Côtes d'Armor) where sporophytes had been discovered for the first time, the fronds were growing further downstream at three close, though distinct, locations; the recorded sporophytes (30, 30 and 20, respectively) were flattened against big boulders, at about 10cm above the running water in well-shaped areas sheltered by dead trees. In the second natural habitat (Saint-Servais, Morbihan), the fronds of *T. speciosum* were hanging from the ceiling of a 25cm deep and wide, 20cm high small alcove where the river turns into a small cascade. It is worth noting that, regardless of the presence of a stream, all the sites with fern sporophytes and or gametophytes were always wet due to abundant leaching waters likely to be responsible for major inputs of minerals to the plant localised in small places with generally little humus. Indeed, the species developed on very thin layer of organic substratum and even directly on the rock. Our observations also demonstrated that, in coastal caves, the fern often appears as gametophyte attached to the ceiling in sites never covered by sea at high tide. Whenever the caves had a certain height, the green clumps were evident on vertical walls located on the sea cliff, in order to avoid flooding by the seawater. This suggests that the gametophytes of Killarney Fern cannot tolerate repeated contact with salt water. Such caves are likely to be submitted to intense sea spray, but as running fresh water was also observed in gametophyte-sheltering caves, salt is probably washed away.

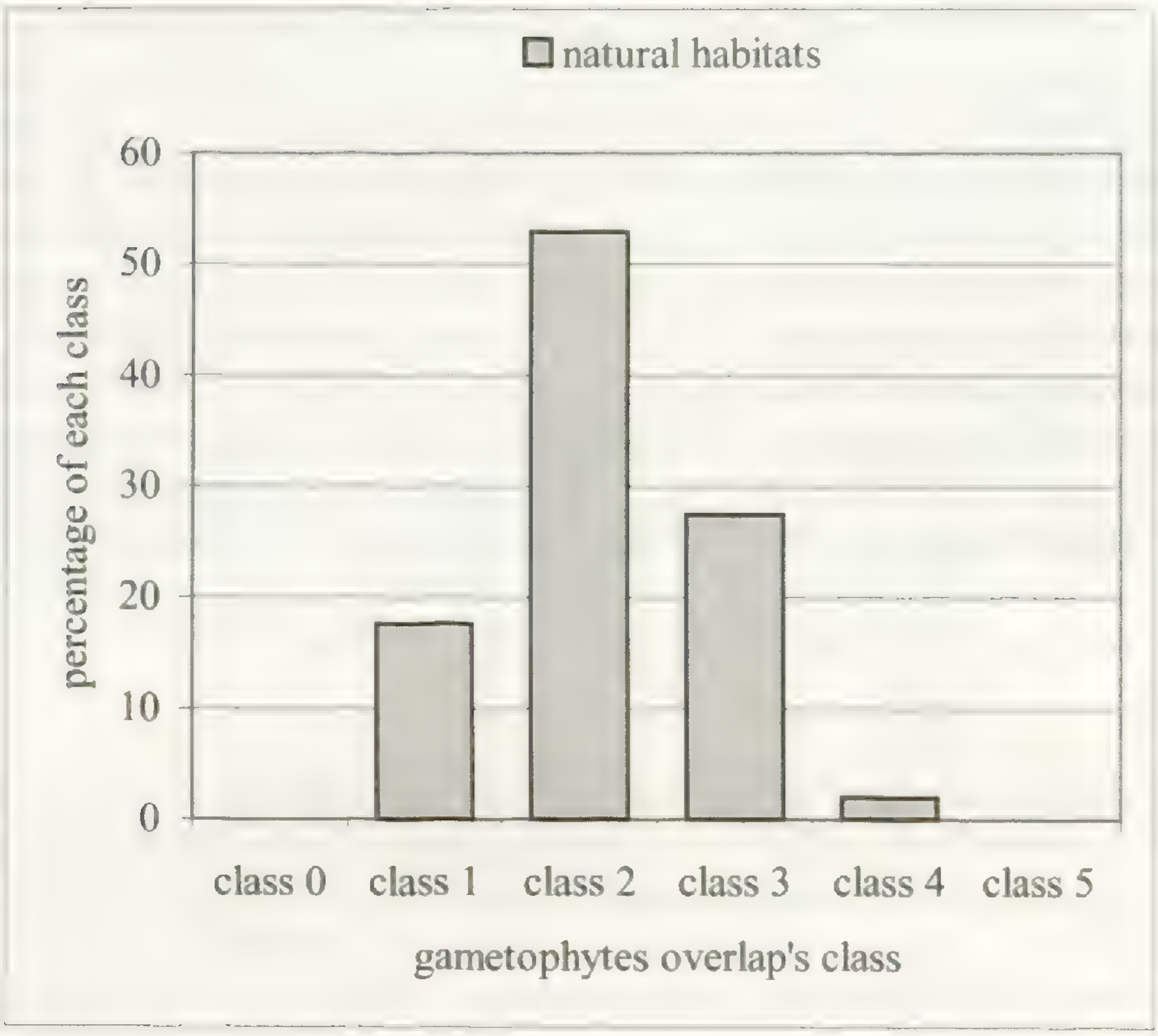


Figure 4a. Percentage of gametophytes overlap class in natural *Trichomanes speciosum* Willd. sites in the Massif Armoricain.

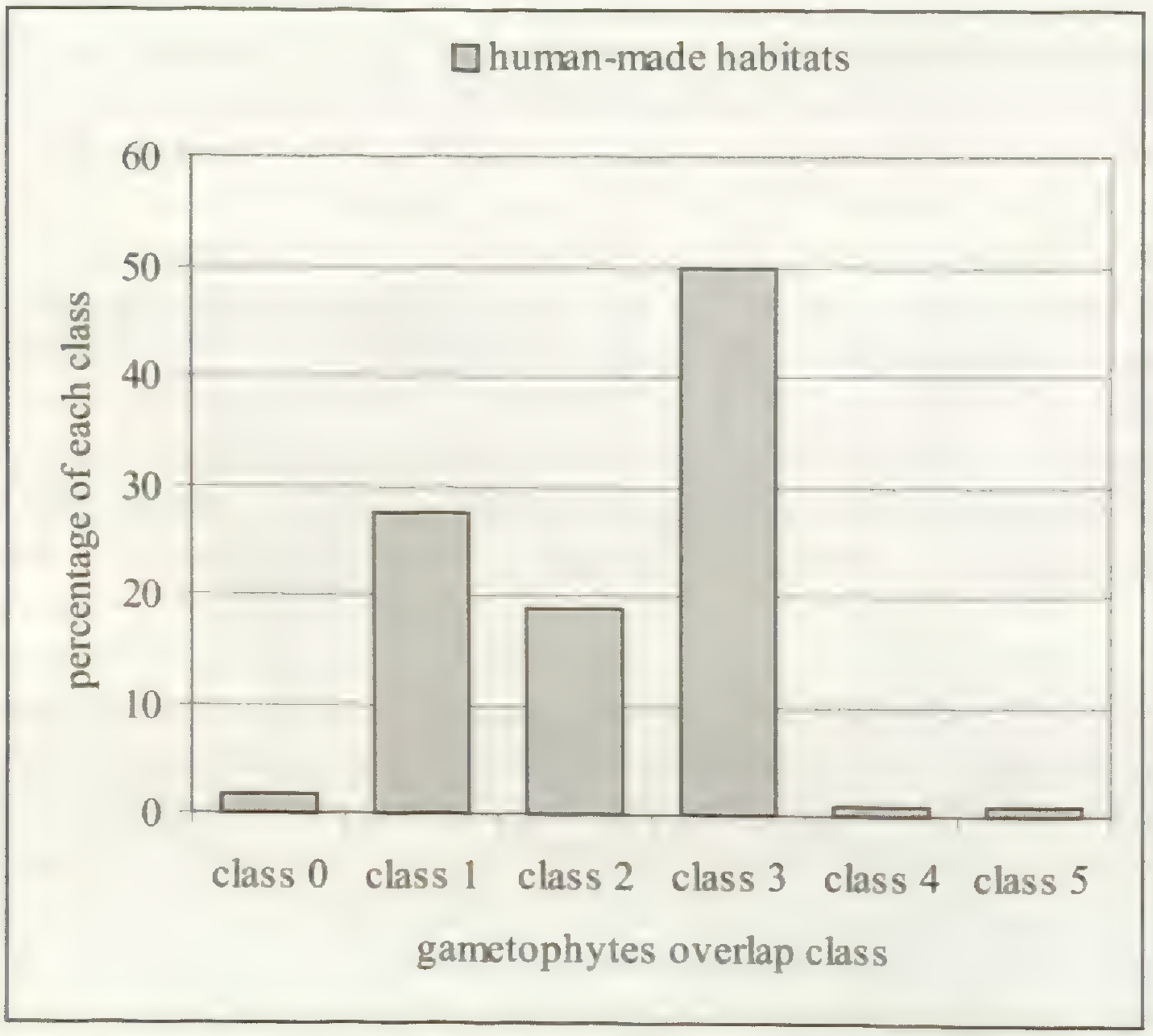


Figure 4b. Percentage of gametophytes overlap class in human-made *Trichomanes speciosum* Willd. sites in the Massif Armoricain.

Numerous pteridophytes, e.g. *Asplenium adiatum-nigrum* L., *A. billotii* F.W. Schultz, *A. scolopendrium* L., *A. trichomanes* L., *Blechnum spicant* L., *Dryopteris dilatata* (Hoffm.) A. Gray, *Dryopteris filix-mas* (L.) Schott, *Pteridium aquilinum* L., *Hymenophyllum tunbrigense* (L.) Sm. and *Dryopteris aemula* (Ait.) Kuntz, were observed at the natural or man-made sites of *T. speciosum*. Under tree cover, mainly composed of *Fagus sylvatica* L. and *Quercus robur* L., the herbaceous layer was usually dominated by *Luzula sylvatica* (Huds.) Gaud. in association with chamaephytes *Vaccinum myrtillus* L. and *Calluna vulgaris* (L.) Hull. Along the coast, *Asplenium marinum* L. was often observed at the entrance of caves sheltering gametophytes of Killarney Fern. Whatever the kind of habitat, bryophytes were the predominant plant species in the vicinity of both stages of *T. speciosum*.

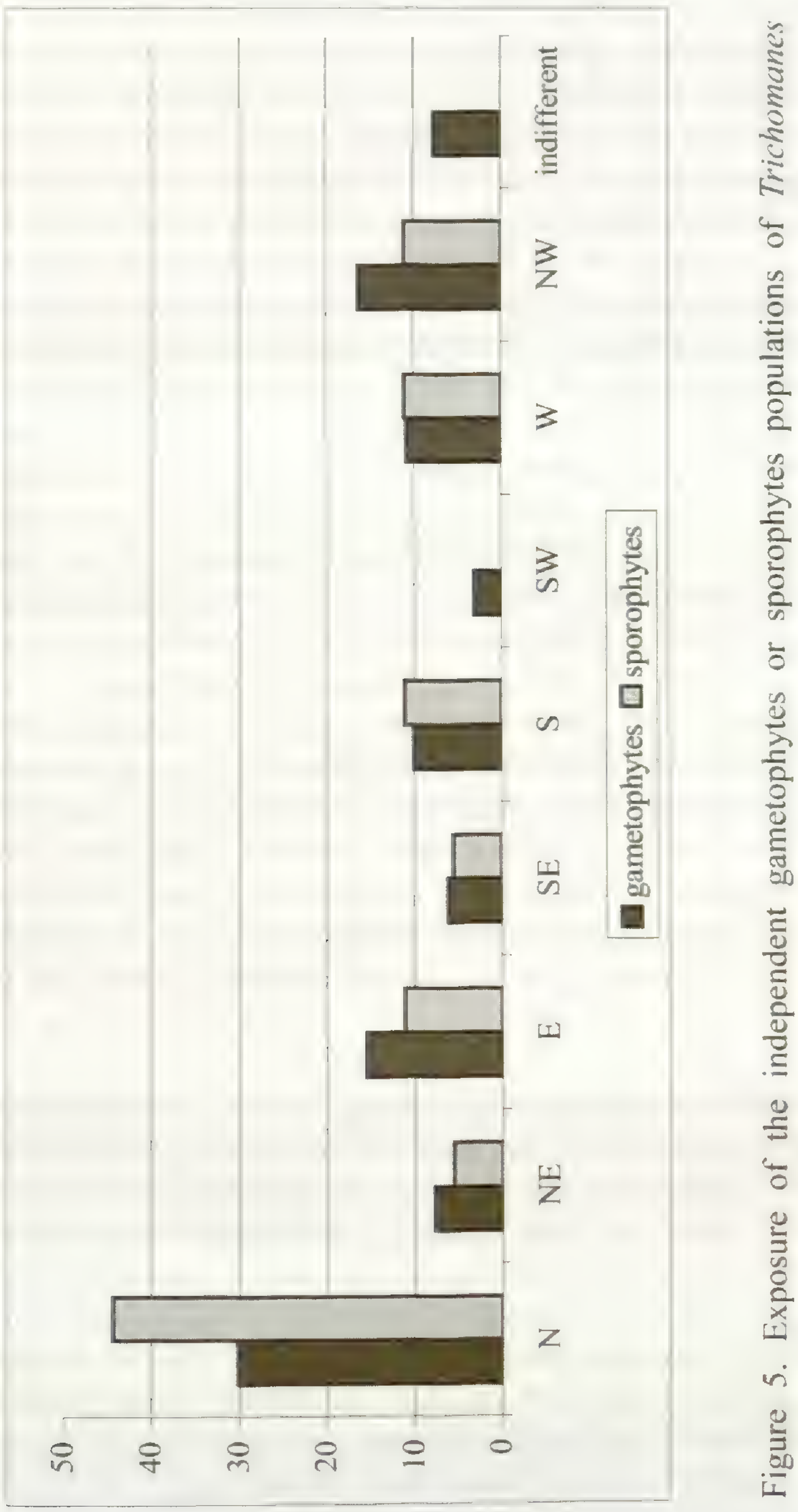


Figure 5. Exposure of the independent gametophytes or sporophytes populations of *Trichomanes speciosum* Willd. in the Massif Armoricain.

Occurrence of tiny sporophytes of *Trichomanes speciosum* across the Massif Armoricain

Tiny sporophytes, less than 0.5cm long, were observed under the binocular microscope (x 10), in samples from six sites in Morbihan, *i.e.* the quarry of *Haut-Sourdéac* (Glénac) and the wells of *Haut-Roussimel* (Glénac); *Vieille Ville* (Taupont), *Lestun* (Cournon), *Saint-Armel* (Bubry), *Croix-Piguel* (Saint Martin), 3 sites of Finistère, *i.e.* woodland outcrops of *Chapelle-Ruinée* (Roche-Maurice), *Stangala* (Ergué-Gaberic), *Le Gouffre* (Huelgoat) and in one site of the Côtes d'Armor in *Vallée du Léguer* (Ploubezre). Thus, in north-western France, some populations of gametophytes seem able to produce sporophytes, but they are so small that they are invisible to the naked eye.

DISCUSSION

Wells still constitute the major habitat of Killarney Fern in north-western France, since they account for 67% of the sites across the Massif Armoricain. The preponderance of *T. speciosum* in this area is related to the preservation of numerous traditional wells. Following the identification of fronds of Killarney Fern in a well in 1948, there has been a systematic inspection started in the 1950s in the surroundings of this first well (J. Moisan, personal communication, 2003). Such a complete exploration has raised the number of sporophyte-sheltering wells to 178 across the Morbihan. One should note that at that time, no gametophyte of the species had been recorded, nor any similar study been conducted elsewhere in France. However, the updated data clearly show that the species is also well-represented in natural habitats, even though it consists mainly of gametophyte populations. They also indicate that the distribution area of the gametophyte is significantly greater than that of the sporophyte (129 and 36 sites, respectively). This observation corroborates those made in the Basque Country where a systematic search for prothallial filaments, totally unknown at this date in this geographical area, was conducted in 2002 (Loriot *et al.*, 2002). Thanks to the criteria defined above for the characterisation of the gametophyte ecology across the Massif Armoricain, the targeting on Basque Country maps of sites liable to shelter the Killarney Fern, allowed fruitful prospecting. The characteristic green clumps were, indeed, discovered in places registered as sporophyte habitats; but, gametophytes of *T. speciosum* were also identified in many other sites with no previous record of the fern. Since then, searches have been targeted to sites considered as potentially favourable to the development or presence of independent gametophytes; they recently led to the report of three new localities (Blanchard *et al.*, 2003). Thus, in the Massif Armoricain and Basque Country, the gametophyte sites are more numerous than the sporophyte ones. This observation agrees with the European distribution area of the fern (Stace, 1997).

Concerning the abundance of gametophytes, the largest areas of overlap were always identified in poorly illuminated sites with large walls; this lack of light prevents other plant species, *e.g.* the bryophytes, to compete with the Killarney Fern. Such conditions are typically found in wells and other types of man-made habitats like quarries, tunnels,etc.; this explains why the present study evidenced a class-3 abundance of the fern in 50% of them. In natural habitats, such a growth was mainly observed in coastal caves; on the other hand, class-2 abundance was principally noticed in forested habitats (53%) where the numerous small and deep cracks of rocky outcrops or woodland boulders, free of inter-species competition sheltered scattered clumps of independent gametophytes.

The recent and first observation, in natural habitats, of two new populations of sporophytes is a major finding. But it must not hide the significant drop in the number of sporophyte-sheltering wells across the Massif Armoricain. Indeed, most of the wells that historically sheltered this fern have been closed, which resulted in the disappearance of fronds because of a lack of light and humidity as evidenced by their brown and withered aspect. It is worth recalling that, since the 1950s, 81% of the sporophyte populations have been destroyed. Moreover, among the few wells still open, only nine of them house a luxurious population of sporophytes (with more than 100 fronds); in the others, the number of fronds ranges within two and 30. The maintenance of the fern in wells is undoubtedly related to their use: when the water is drawn from the well, the fronds are sprinkled.

Thus one must not consider *T. speciosum* as abundant across the Massif Armoricain despite the 165 recorded sites; its conservation state is undoubtedly alarming, since a third of the 34 inspected wells are endangered in the immediate short-term. Failing to implement conservation measurements would cause the disappearance of this species from Central Brittany within a few years. This is why the Conservatoire Botanique National de Brest has produced an informative leaflet, "*Trichomanes speciosum*, a rare plant to safeguard", for owners of wells sheltering the precious fern at any stage of its life-cycle. Owners are requested to water the fern regularly to mimic the ancestral gesture. Moreover, so as to eliminate the deleterious closing of many wells with pieces of metal or wood, from an initiative of CBN Brest specifically designed grids are being installed on wells by the technical services of the local town councils. In a near future, it is planned that owners of a piece of forest or coastal fringe will be informed of the presence of the fern and educated through a further leaflet.

CONCLUSION

The conservation of the identified populations of independent gametophyte is essential because of their inherent interest but also their potential to eventually produce sporophytes through sexual reproduction. Indeed, in north-western France, the "independent gametophyte" has the capability to form tiny sporophytes. The scarcity of these tiny sporophytes and the small size of fronds are both remarkable. Our difficulty lies in the definition of conditions favourable to the development of the adult stage from these small sporophytes. Ongoing experiments focus on analysis of environmental parameters to determine whether these are responsible for arresting the growth of the tiny sporophytes at a juvenile stage in most of the natural habitats across the Massif Armorican by inducing physiological deficiency.

ACKNOWLEDGEMENTS

The authors thank the Conseil Régional de Bretagne and the DIREN Bretagne for the financial support of this study. They are grateful to Pr. Frédéric Bioret (IUEM, Plouzané) for commenting on the manuscript, and Marie-Paule Friocourt (UBO, Brest) for reviewing the use of English.

REFERENCES

ALLORGE, V., ALLORGE, P. 1941. Bulletin de la Société Botanique de France. Les ravins à fougères de la corniche vasco-cantabrique, 8: 93-111.

BLANCHARD, F., LAMOTHE T. & LORIOT, S. 2003. Contribution à la répartition du gamétophyte indépendant de *Trichomanes speciosum* Willd. (*Hymenophyllaceae*,

Pteridophyta) dans le Pays basque (Pyrénées-Atlantiques). Le Monde des Plantes. 481: 15-17.

BIORET, F. & KERBIRIOU C. 1994. Catalogue des espèces et des habitats de la Directive Habitats présents en Bretagne. Directive 92/43 CEE du conseil du 21 Mai 1992. Brest, 232 p.

BIZOT, A. 2000a. *Trichomanes speciosum* Willd. découvert sur le versant méridional des Vosges (Haute-Saône). Le Monde des Plantes, 469: 7.

BIZOT, A. 2000b. Découvertes de trois nouveaux taxons de Ptéridophytes dans les Ardennes. Bull. Soc. Hist. Nat. des Ardennes, 90: 67-74.

BIZOT, A. 2004. Note chorologique complémentaire concernant les gamétophytes de *Trichomanes speciosum* Willd. Le Monde des Plantes, 482: 6-7.

BOODLE, L.A. 1900. Comparative anatomy of the *Hymenophyllaceae*, *Schizeaeceae* and *Gleichniaceae*. I. On the anatomy of *Hymenophyllaceae*. Ann. Bot. London. 14: 455-496.

BOUDRIE, M. 2001. Première découverte de gamétophytes de *Trichomanes speciosum* Willd. (Hymenophyllaceae, Pteridophyta) dans le Massif Central français. Bulletin de la Société Botanique du Centre-Ouest. Nouvelle série, 32: 73-78.

CUSSET, G. 1997. Botanique. Embryophytes. Ed. Masson. Paris 512p.

DES ABBAYES, H. 1971. Flore et végétation du Massif Armoricain 1: Flore vasculaire. P.U.B S^{t} Brieuc, 1226 pp.

EUROPEAN COMMUNITY 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Brussels.

FARRAR, D.R. 1967. Gametophytes of four tropical fern genera reproducing independently of their sporophytes in the Southern Appalachians. Science, 155: 1266-1267.

FARRAR, D.R. 1985. Independent fern gametophytes in the wild. Proceedings of the Royal Society of Edinburgh, 86: 361-369.

JERMY, C. & CAMUS J. 1991. The illustrated field guide to ferns and allies plants of the British Isles. 194 S. Natural Hist. Mus. Publ. London. 208p.

JEROME, C., RASBACH, H. & RASBACH, K. 1994. Découverte de la fougère *Trichomanes speciosum* (Hymenophyllaceae) dans le massif vosgien. Le Monde des Plantes, 450: 25-27.

JEROME, C. & BIZOT, A. 2001. La réserve de Biosphère des Vosges du Nord: un paradis pour les gamétophytes de la fougère *Trichomanes speciosum* Willd. Ann. Sci. Bios. Vosges du Nord-Pfâlzerwald, 9: 63-72.

JOVET, P. 1933. Le *Trichomanes radicans* (Sw.) et l'*Hymenophyllum tunbrigense* (Sm.) en Pays Basque français. Bulletin de la Société Botanique de France, 80: 797-809.

KRUKOWSKI, M. & SWIERKOSZ, K. 2002. Sandstone, landscapes: diversity, ecology and conservation. *In*: Newsletters Central and eastern Europe. IUCN European programme newsletter. p17.

LORIOT, S., BLANCHARD, F., LAMOTHE, T., COTTIGNIES, A. 2002. Présence du gamétophyte indépendant de *Trichomanes speciosum* Willd. (*Hymenophyllaceae*) dans les vallées du Pays Basque. Premières données concernant sa distribution dans les Pyrénées-Atlantiques. Le Monde des Plantes n° 477 p 23.

LOUIS-ARSENE 1953. *Trichomanes speciosum* Willd. en Bretagne. Bulletin de la Société Botanique Française, 100: 6.

MAKGOMOL, K. & SHEFFIELD, E. 2001. Gametophyte morphology and

ultrastructure of the extremely deep shade fern, *Trichomanes speciosum*. New Phytologist, 151: 243-255.

OLIVIER, L., GALLAND, J.P. & MAURIN, H. 1995. Livre Rouge de la Flore Menacée de France I: les espèces prioritaires. Ministère de l'environnement. Muséum National d'Histoire Naturelle. Conservatoires Botaniques Nationaux. Paris. 486 p + annexes.

PAGE, C.N. 1997. The ferns of Britain and Ireland, edn 2. Cambridge: Cambridge University Press. 450pp.

PAGE, C.N., DYER, A.F., LINDSAY, S. & MANN, D.G. 1992. Conservation of pteridophytes: the *ex situ* approach. *In* IDE, J.M., JERMY, A.C. & PAUL, A.M (Eds).: Fern Horticulture: past, present and future perspectives, pp. 269-278.Intercept, Andover.

POUX, L., PHILIPPON, D. & PRELLI, R. 2003. Bilan des découvertes intéressantes de l'année 2002 dans les Côtes d'Armor. Erica 17, p 81. Ed. Conservatoire Botanique National de Brest, France.

PRELLI, R. & BOUDRIE, M. 1992. Atlas écologique des Fougères et plantes alliées. Illustration et répartition des Ptéridophytes de France. Ed. Lechevalier, 272 p.

PRELLI, R. 2002. Les fougères et plantes alliées de France et d'Europe Occidentale. Ed. Belin. Paris. 431p.

RASBACH, H., RASBACH, K., JEROME, C. & SCHROPP, G. 1999. Die Verbreitung von *Trichomanes speciosum* Willd. (Pteridophyta) in Südwestdeutschland und in den Vogesen. Carolinea, 57: 27-42.

RATCLIFFE, D.A., BIRKS, J.B. & BIRKS, H.H. 1993. The ecology and conservation of the Killarney fern *Trichomanes speciosum* Willd. in Britain and Ireland. Biological Conservation, 66: 231-247.

RICH, T.C.G., RICHARDSON, S.J. & ROSE, F. 1995. Tunbridge filmy-fern *Hymenophyllum tunbrigense* (Hymenophyllaceae: Pteridophyta) in South East England in 1994/1995. Fern Gazette, 15: 51-63.

RIVIERE, G. 1999. Les ptéridophytes du Morbihan. Le Monde des Plantes, 465: 22-23.

RUMSEY, F.J., SHEFFIELD, E. (1996) Inter-generational ecological niche separation and the "independent gametophyte" phenomenom. In: CAMUS J.M., GIBBY M. & JOHNS R.J. (Eds), Pteridology in perspective, pp.563-570. London, UK: Royal Botanic Gardens, Kew.

RUMSEY, F.J., JERMY, A.C. & SHEFFIELD, E. 1998. The independent gametophyte stage of *Trichomanes speciosum* Willd (Hymenophyllaceae) the Killarney Fern and its distribution in the British Isles. Watsonia 22: 1-19.

STACE, C. 1997. New Flora of the British Isles. Cambridge University Press. 1226 p.

STOCKEY, A.G. 1940. Spore germination and vegetative stages of the gametophytes of *Hymenophyllum* and *Trichomanes*. Bot. Gaz., Chicago, 101: 759-790.

TRYON, A.F. & LUGARDON, B. 1990. Spores of the Pteridophyta: Surface, wall structure and diversity based on electron microscope studies. Springer-Verlag, New York, 648 p.

WIGGINTON, M.J. 1999 British Red Data Books: 1. Vascular Plants. 3rd edition. JNCC ed. 200 p.

BOOK REVIEW

THE FIRST BOTANICAL COLLECTORS IN NEPAL – The Fern collections of Hamilton, Gardner and Wallich – lost herbaria, a lost botanist, lost letters and lost books somewhat rediscovered.

continued from page 314

be hoped that it can be rescued before its destruction by the ravages of the Bengal climate.

Given the great virtues of this book it seems churlish to nitpick, but as has already been hinted, there are dangers with such a personal style, and it could have been judiciously edited without destroying its character: minor personal rants such as that over Fraser-Jenkins's own 'author abbreviation' could usefully have been lost. There are remarkably few errors of fact or typography, but Hodgson was not knighted and Thomas Thomson did not take back a set of EIC specimens to Calcutta in 1855. The author's highly approving view of Wallich's personality is also debatable.

These are but minor quibbles in what is a fascinating and important work. The publishers are to be congratulated on taking on a work that it would have been hard to publish in Britain. It is a handsome slim volume, on excellent paper, with good colour reproduction in the interesting section of plates, and much better bound than many modern Indian books.

H.J. Noltie

A MOULDING METHOD TO PRESERVE TREE FERN TRUNK SURFACES INCLUDING REMARKS ON THE COMPOSITION OF TREE FERN HERBARIUM SPECIMENS

T. JANSSEN[1, 2]

[1] Muséum National d'Histoire Naturelle, Dépt. Systématique et Evolution, UMR-CNRS 5202 / USM 602, CP39, 57 rue Cuvier, F-75231 Paris cedex 05, France
[2] Georg-August-Universität Göttingen, A.-v.-Haller-Institut für Pflanzenwissenschaften, Abt. Spezielle Botanik, Untere Karspüle 2, 37073 Göttingen, Germany; (Email: thomas.janssen@bio.uni-goettingen.de)

Key words: collection, Cyatheaceae, Dicksoniaceae, field annotation, herbarium specimen, imprinting, large ferns, moulding, silicone, trunk surface.

ABSTRACT

Ferns with a tree habit are mainly found in the families Cyatheaceae and Dicksoniaceae. Together, they constitute a diverse pantropical group of common to locally dominant elements of tropical floras, especially in montane forests. Many species have very restricted distribution ranges and some are highly threatened. Taxonomic understanding of these ferns, a prerequisite to successful establishment of conservation strategies, is hampered by the disparate and often insufficient quality of available herbarium material. During recent fieldwork, a time-efficient scheme has been developed permitting to maximize the information content of tree fern collections. A simple non-destructive silicone moulding method to preserve important characters of the trunk surface is introduced. A standardized annotation sheet as a field book supplement is provided along with a summary sheet of the presented collecting approach for quick reference in the field. Standardized annotations and associated collections such as stem moulds greatly augment the value of the specimen.

INTRODUCTION

Tree ferns constitute a significant fraction of plant diversity in the world's (sub-) tropical forests with approximately 650 described species (Large & Braggins, 2004; Kubitzki, 1990). The tree fern lineage comprises the families Cyatheaceae and Dicksoniaceae as well as Hymenophyllopsidaceae, Lophosoriaceae, Metaxyaceae, and Plagiogyriaceae not all of which have a tree like habit (Korall et al., 2006; Pryer et al., 2004). The scaly tree ferns (Cyatheaceae) account for more than 90% of the species diversity in the tree fern lineage (Kubitzki, 1990). The status of some of these families, especially the polyphyletic Dicksoniaceae, has been re-evaluated recently, but the names used here follow Kubitzki (1990). Besides true tree ferns, some species of Blechnaceae, Osmundaceae, and Thelypteridaceae can be designated as ferns with a tree-like habit. For the purpose of this paper, the term "tree ferns" is used to designate ferns with an erect aerial stem (generally referred to as "caudex", "rhizome", or "trunk") reaching a height of three meters in most and up to 20 meters in some species, in combination with generally big leaves with a lamina up to five meters and petiole diameters up to five centimeters (Large & Braggins, 2004; Moran, 2004).

During ongoing revision work of the tree fern family Cyatheaceae in the Western

Indian Ocean (Madagascar and adjacent islands) the author was confronted with problems confirming the timeliness of earlier statements that tree ferns have been and often are still being collected in non-representative fragments (e.g., Johns, 2000; Brownsey, 1985; Stolze, 1973; Holttum, 1957a). Although a considerable amount of herbarium material of tree ferns exists, the quality and usefulness of specimens varies enormously. Most ancient collections are fragmentary, often consisting of a single pinna (Fig. 5A). More recent collections are of disparate quality ranging from complete collections displaying all necessary information on the label via complete specimens lacking sufficient annotation to unannotated fragmentary specimens of unknown origin.

Tree ferns present a wide array of morphological features that will not fit on a herbarium sheet (Fig. 1). In order to document the morphology of a tree fern as completely as possible, herbarium specimens may be accompanied by written field observations and / or complemented by supplementary material. This paper will shortly review suggestions concerning the composition of tree fern herbarium specimens that have already been discussed elsewhere (Roux, 2001; Johns, 2000; Croft, 1999; Brownsey, 1985; Stolze, 1973; Holttum, 1957a). It will focus on annotations and supplementary collections that may augment the value of the specimens.

The suggestions presented herein have been elaborated based on experiences during extensive fieldwork in 2004-2005 focused on the collection of tree ferns in Madagascar and adjacent islands. Madagascan endemic Cyatheaceae are restricted to primary forests (Rakotondrainibe, 2003; Koechlin et al., 1974; and references therein) and generally under high unspecific, e.g. habitat destruction (Ingram & Dawson, 2005; Brown & Gurevitch, 2004; Brooks et al., 2002; Du Puy & Moat, 1998), and specific, e.g. exploitation of trunks for the fabrication of flower pots and fences (Ranarijaona, 1993), anthropogenic pressure. The availability of unambiguously determinable herbarium specimens and well-established taxonomic entities is seen as a prerequisite for suggesting conservation priorities as well as for the recognition of rare and possibly endangered taxa (Isaac et al., 2004; Mace, 2004; Willis et al., 2003; McNeely, 2002; Schatz, 2002). Plant collections in the form of herbarium specimens are still one of the most important instruments to document plant diversity (Schatz, 2002). It is desirable that the recommendations given herein will contribute to our understanding of the taxonomy of the tree fern lineage by increasing the number of available unambiguous specimens.

A SILICONE MOULDING TECHNIQUE TO PRESERVE TRUNK SURFACES

The moulding technique applies to tree ferns with caducous petiole bases and naked trunks, i.e. with the leaf scars exposed (Fig. 1a). The relief, spinescence, form and disposition of leaf scars as well as the surface structure of the trunk exhibit taxonomically valuable interspecific variation. Although helpful, photographic documentation is, with respect to contrast and interpretability, often not sufficient to document fine three-dimensional structures. Cutting parts of the trunk surface or whole trunks irreversibly damages the plant and is not reconcilable with collecting tree ferns in compliance with conservation requirements.

A convenient way to preserve tree fern trunk surface structures without inflicting damage on the plant is the use of a silicone moulding technique (Fig. 2). Fast hardening, non-toxic moulding silicones are available from suppliers of dentist's materials. Excellent experiences have been had with the two component silicone "panasil® putty fast set" from Kettenbach Dental. Moulding techniques are, among other applications,

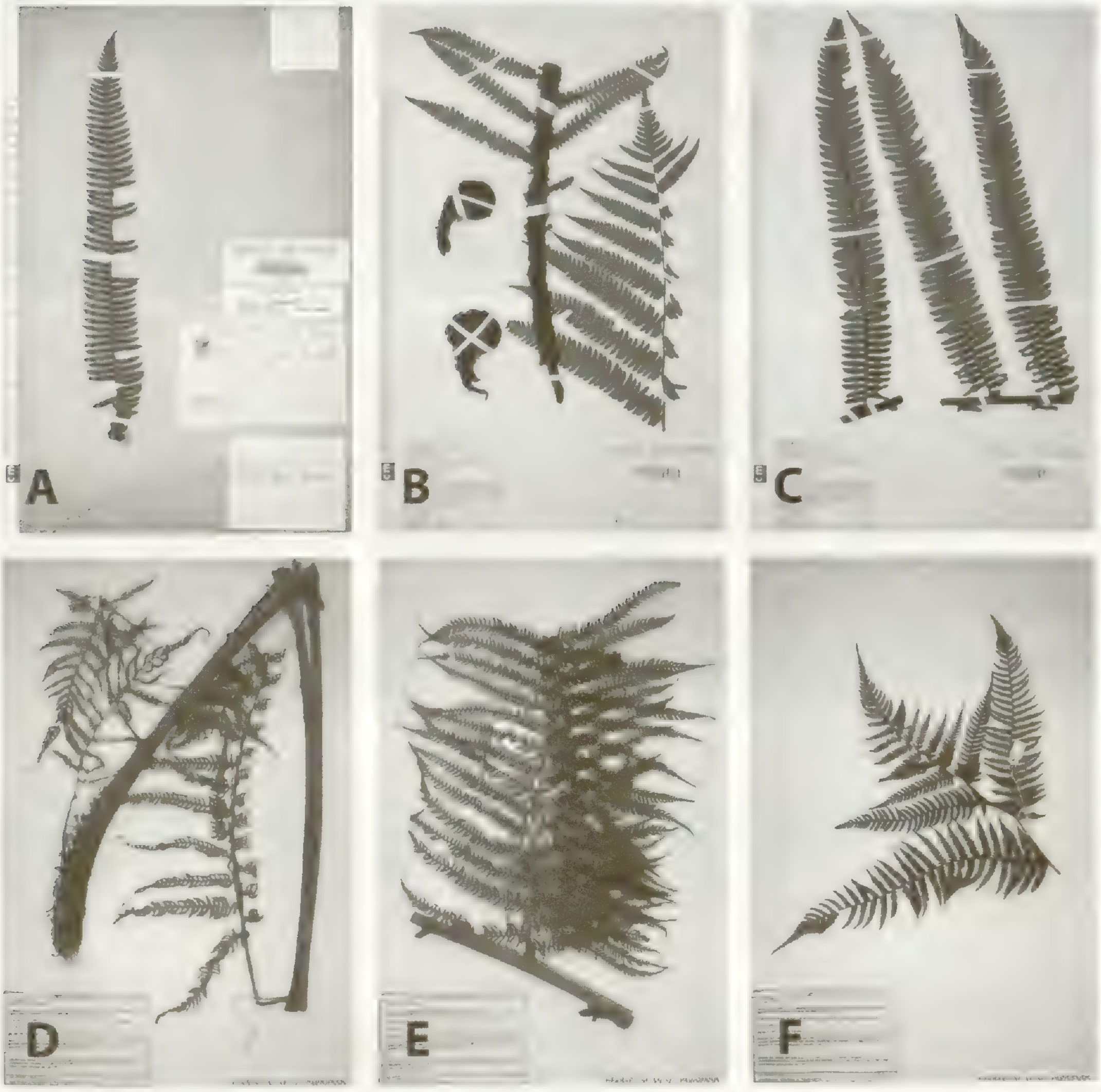


Figure 5. Herbarium specimens of tree ferns. (A) The holotype of *Cyathea orthogonalis* Bonap. from Madagascar consists of a single pinna (Baron 6126, P). The species is distinguished from closely related taxa with difficulty in the absence of stipe scales and cannot adequately be described from such fragmentary material. (B, C) A complete collection of *Cyathea orthogonalis* Bonap. (Rasolohery 628, P), a medium sized tree fern. The petiole has been collected from the base up to the first pinna pairs. The lamina apex as well as two croziers (unfolding fronds, bearing young scales) have been collected (B). Middle pinnae are collected with a fragment of the rachis. Pinnae on one side of the rachis have been pruned away leaving a short fragment attached to the rachis (C). (D-F) Herbarium specimen of the large sized tree fern *Cyathea hildebrandtii* Kuhn. (Janssen 2433, P) mounted on three sheets. The petiole has been collected from the base to the first pinna pair, halved lengthwise and folded to fit the sheet (D). One pinna from the middle part of the frond has been collected with a sufficiently long rachis fragment and folded to fit the sheet (E). (photos: MNHN, Paris)

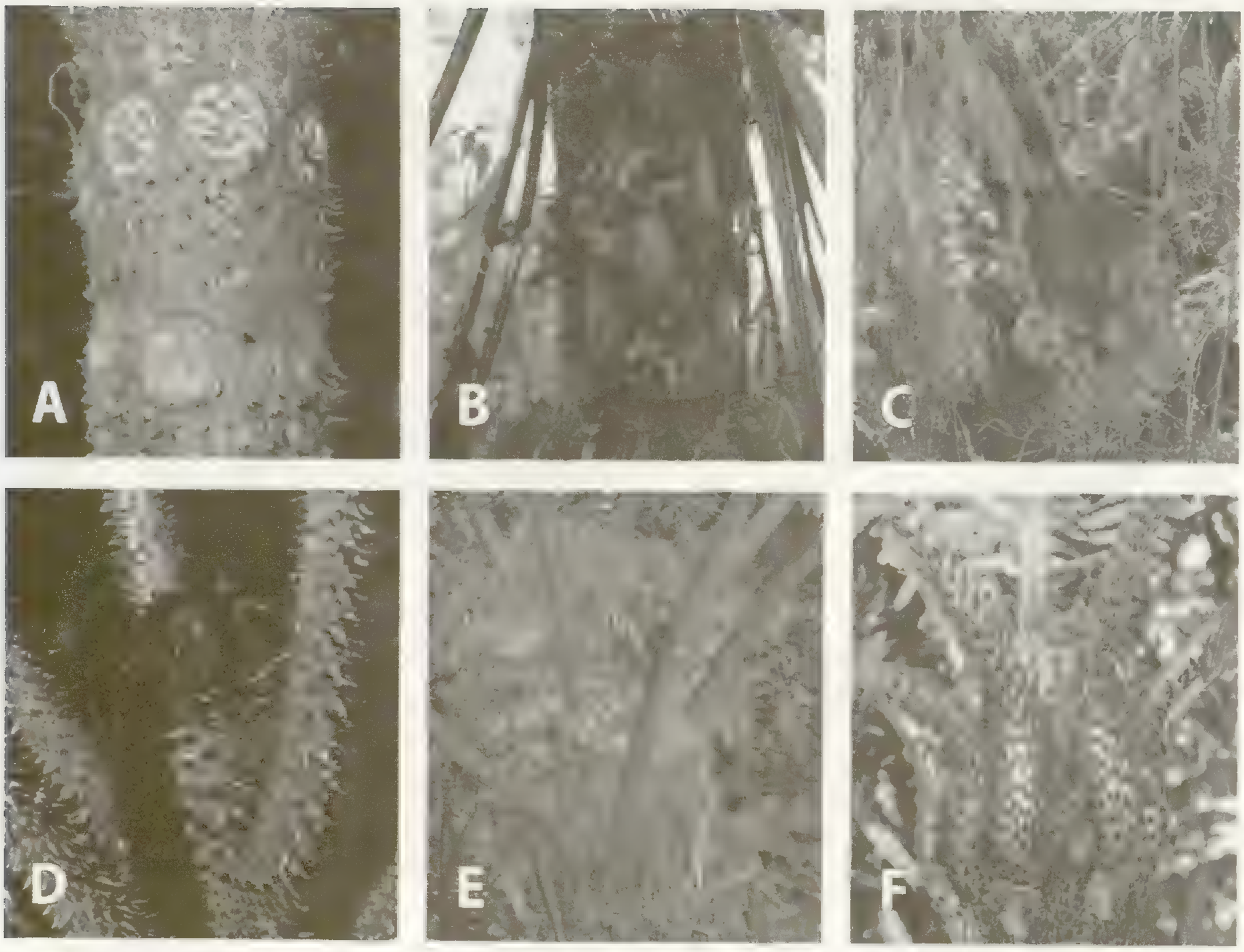

A
B
C
D
E
F
G
H
I
J
K
L

Figure 1 (left). Morphological characters of tree ferns that are of taxonomic value but difficult to preserve in herbarium specimens. *Trunk surface:* The trunk may be naked (A; *C. auriculata* Tardieu) or covered by persistent bases of fallen petioles (B; *C. lastii* Baker). *Ramifications*: Being simple in most species, the trunk of some species is frequently ramified (C; *C. dregei* Kunze). *Trunk apex:* The trunk apices are highly diverse with respect to indument and the arrangement of petioles that is often characteristic for the species: (D) *C. ligulata* Baker with spiny indument on the petioles and on the well-visible apex; (E) *C. fadenii* Holttum with the apex completely covered by abundant aphlebia; (F) *C. decrescens* Mett. ex Kuhn with the apex completely covered by crowded petiole bases bearing distinct deltoid scales. *Habit*: The habit of the plant allows easy differentiation of some species and crown shape should always be sketched or photographed (G, *Cyathea sp.*; H, *C. borbonica* Desv.). *Lamina*: Where appropriate, lamina shape and distribution of fertile pinnae should be documented (I; *Cyathea sp.*). *Aphlebia*: Aphlebia (J; *C. bullata* (Baker) Domin) and aphlebioid pinnae (K; *C. hildebrandtii* Kuhn) are present in some species. *Aerophores:* Aerophores, usually in one to several rows on either side of the petiole are usually clearly visible on fresh material, but are difficult to see on herbarium specimens (L; *C. hildebrandtii* Kuhn). (photos A, D, E, F, K, L: Germinal Rouhan, MNHN)

Figure 2. Moulding the trunk surface of a tree fern. See text for explanation. (photos: Germinal Rouhan, MNHN)

widely used in anthropology, criminology and zoology. In botanical research they have been applied to preserve surface structures for microscopical analysis (Kwiatkowska & Dumais, 2003; Green et al., 1991; Hernández et al., 1991; Sheffield et al. 1991; Tiwari & Green, 1991; Williams, 1988; Jörg, 1965; Deckart, 1959; Loske, 1959), in moulding plant fossils (e.g. Feix & Howorka, 1965), or in making replica of perishable specimens (e.g. Mortemore, 1968).

In the first step, the trunk surface of the tree fern is cleaned of epiphytes and litter with a hard brush, but care should be taken during this process to preserve protuberant surface structures. In the second step, equal amounts of the non-toxic two-component silicone are mixed by kneading and then applied to the trunk surface with the heel of the hand. The mould should include at least two or three petiole scars and be thick enough to cover all surface structures (e.g. spiny structures of the scar rim). Care should be taken to apply sufficient pressure to fill all cavities. Before the silicone has set, the collection number should be engraved directly on the outside of the mould with a pen or small stick in order to provide a permanent means of identification of the mould. The mould will harden in two to three minutes (more quickly in cold weather) and even under wet conditions. It can then easily be pulled away from the surface.

This method is easy to use even by untrained plant collectors and yields extremely detailed surface moulds (Fig. 3), which provide suitable information for taxonomic revision. The moulds remain flexible, which facilitates transport under field conditions. Moulds can be stored in bags on the herbarium sheet of the specimen or, when large and heavy, be stored in a separate collection cross-referencing labels with associated herbarium sheets. No data are available on the long-term storage performance of the silicone used, but general properties of silicones promise that the moulds will remain

Figure 3. Taxonomic importance of trunk surface characters. As illustrated by these examples, substantial morphological diversity can be found in the trunk surfaces of tree fern species with caducous petiole bases. Moulding the trunk surface with silicone allows the preservation of structures such as papillae or squaminate spines as well as morphology and arrangement of leaf scars including associated spines or orifices. (A) *Cyathea lastii* Baker (Janssen 2381, P) with large oval spirally arranged scars and densely muricate surface. (B) *Cyathea auriculata* Tardieu (Janssen 2508, P) with small rounded pseudoverticillate scars and a sparsely muricate surface. (C) *Cyathea sp.* (Janssen 2802, P) with oval pseudoverticillate scars bearing up to seven strong spines on their lower rim and a smooth surface. (Scale bar is 5cm; photos: MNHN, Paris)

sufficiently constant in form over extended periods of time (Oldfield & Symes, 1996; Feix & Howorka, 1965).

HERBARIUM SPECIMENS OF TREE FERNS

Remarks on the composition of specimens of large ferns, especially tree ferns, have been made by Holttum (1957a), Stolze (1973), Brownsey (1985), Croft (1999), Johns (2000), and Roux (2001). All authors agree in proposing to collect at least one to several middle pinnae of each frond together with a rachis fragment as well as the base of the petiole with scales. Some authors preferred to present a basic collecting approach at the expense of a comprehensive discussion of field annotations while others prefer much more information. In the following section the composition of a complete tree fern specimen is discussed and a simple standardized fill-in data sheet for field annotations is proposed.

Collecting tree ferns

It is assumed that collectors are familiar with general collecting techniques (e.g., Liesner & al., 2006; Bridson & Forman, 2004; Hicks & Hicks, 1978). Tree ferns can be collected without inflicting major damage to the trunk or apex of the plant. Fronds should be cut at their very base, i.e. the junction of the petiole with the trunk. This can be difficult in species with petiole bases appressed to the stem. An excellent tool to achieve this is a (telescopic) collecting pole carrying a hooked blade (Fig. 4). Good experiences have been had with the relatively inflexible marine mesh rods available from Daiwa (www.daiwa.com). These are light to carry and with an extension of 5-6m are long enough to reach the crown of most tree ferns. Other commonly employed collecting poles may serve the same purpose. If no specialized collecting tool is

Figure 4. Collecting tool for tree ferns.

available and provided the specimen is not too tall, then fronds can often be detached cleanly at their base by pulling the rachis or petiole, quickly and with determination, perpendicular towards the soil surface.

Preserving entire fronds by cutting them into pieces yields redundant and encumbering specimens. Of one frond it is sufficient to collect:

1) The petiole from its very base up to the first pair of pinnae (Fig. 5 B, D). If it is very thick it can be halved lengthwise. If possible, it should not be cut, but folded when pressing (Fig. 5 D). Attention should be paid on preserving the diagnostically important scales at the base of the petiole (e.g., Holttum, 1957b).

2) One (Fig. 5 E) to several (Fig. 5 C) pinnae from the widest part of the lamina. A fragment of the rachis (at least to the next pinna which may be pruned away if too large to fit on the sheet) must always remain attached. The pinna(e) on one side of the rachis fragment may be pruned away. If fertile and sterile pinnae are dimorphic, at least one pinna of each type must be included in the herbarium specimen.

3) The apex of the frond (Fig. 5 B, F).

Hence, one tree fern frond equals one specimen. Exceptionally, very large fronds may yield two specimens by halving the petiole lengthwise, collecting two middle pinnae with their respective rachis fragments from the widest part of the frond and complementing one specimen with an apex taken from another frond of the same plant.

Material not essential to, but further enhancing the value of the specimen is discussed in the following paragraph. Its collection will depend on availability and logistic constraints. Caducous petiole scales may be collected in a separate envelope in order to prevent the available scale material from being lost or damaged during subsequent treatment of the specimen. A mould of the trunk surface may be prepared according to the method described above. Lamina fragments dried in silica gel are sources of DNA for molecular phylogenetic analyses. Especially when collecting specimens in alcohol (which usually destroys DNA) and when encountering potentially rare species or working in remote areas special care should be taken to preserve at least one sample in silica gel for each putative taxon encountered. If present, adventitious buds or juvenile plants may add information on characters of the developing scales and frond architecture. Some adventitious roots may be dried for subsequent anatomical studies. A young, unfolding leaf (crozier), generally densely covered by young scales that display best the morphology of the scale margin (Holttum, 1957a, b) could be dried or preserved in alcohol. If possible, the habit, trunk apex and trunk surface at breast height can be photographically documented.

As a result of experiences made during recent herbarium studies and taking into account discussions with herbarium curators, it might be of value to repeat here that when selecting specimens for loan or exchange, all associated material should be sent. In the past, distribution of specimens to other herbaria has frequently resulted in fragmentary duplicates. Although much of the fragmentary material can be determined, its value for revision work is limited. It is better to have complete specimens in a few herbaria than to have incomplete specimens in many herbaria.

Annotating the specimens

Several morphological characters of tree ferns cannot, or only with difficulty can be preserved in herbarium specimens (Fig. 1). Hence, these characters are often neglected in taxonomic revisions. When discriminating species in the field, characters such as habit, trunk surface or trunk apex may aid in identification, but are seldom recorded,

TRUNK

_____ m x _____ cm
(ramifications, adventitious buds, thickened base, dead fronds / rachises persistent) :

TRUNK SURFACE
(colour, surface structure, scales, adventitious roots) :

SCARS

_____ x _____ cm
(form, spines or orifices, arrangement (spiral (number orthostiches?), pseudo-verticillate (number per whorl?)) :

CROWN
(form, angle of fronds, number of fronds, morphology of vegetative point (visible or densely covered by petiole bases, scaly or naked)) :

PETIOLE

diameter : _____ cm
base straight, recurved, sigmoid;
scales (if caducous) :

aerophores (size, colour, disposition) :

basal reduced pinnae (structure, size, number pairs, distance from petiole base, present on all fronds?) :

colour of petiole, rachis, costae (adaxial / abaxial faces) :

LAMINA
shape:

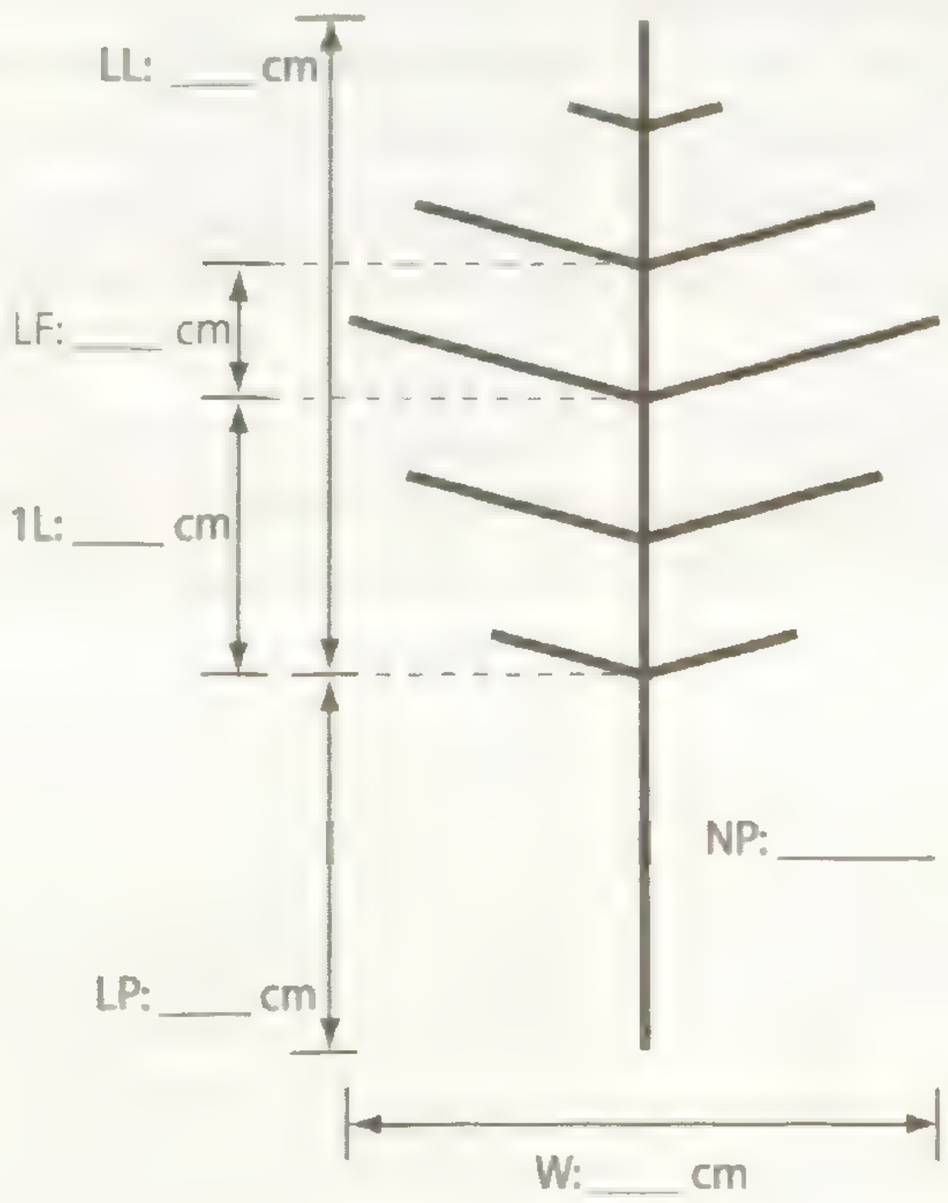
colour adaxial / abaxial :

texture :

basal pinnae conduplicate / reflexed :

base abruptly/gradually reduced :

fertile pinnae (all or part (which?) of frond) :



number and species

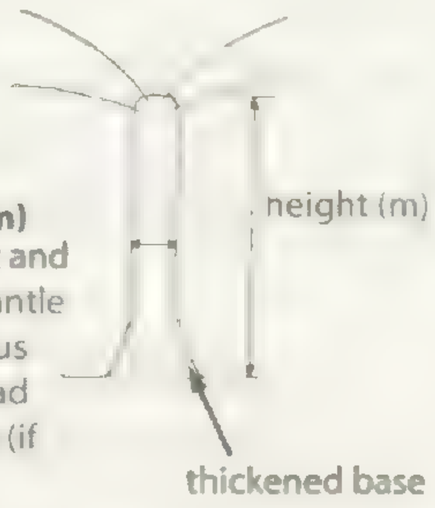
composition of collection

___(___) # fronds (# specimens)
___ photos (__ habit, __ apex, __ trunk, __ other)
☐ silicagel
☐ surface mould
☐ petiole scales
☐ adventitious roots
☐ adventitious buds
☐ croziers
☐ _____

GENERAL REMARKS AND SKETCHES
(habit, scar, frond, ...)

Figure 6. Field data sheet for recording morphological information. This sheet is suggested as a template for data that should be recorded when collecting tree ferns. Terminology and abbreviations are explained on the reference sheet (Figure 7).

TRUNK



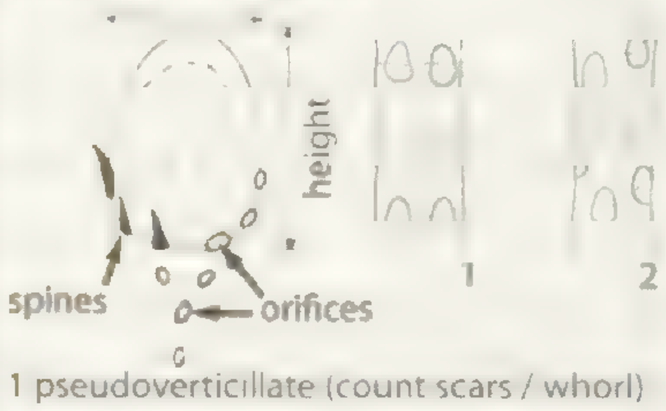
Persistent dead fronds / rachises are hanging from the apex and +/- covering the trunk.

TRUNK SURFACE

- petiole scars are visible or covered by **dead petiole bases**
- muricate, tuberculate, smooth, etc.
- **scaly or naked**

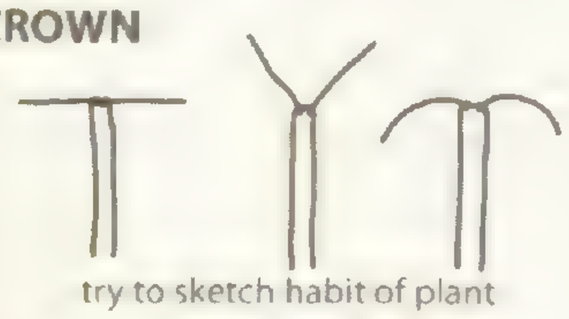
SCARS

width (at widest point)



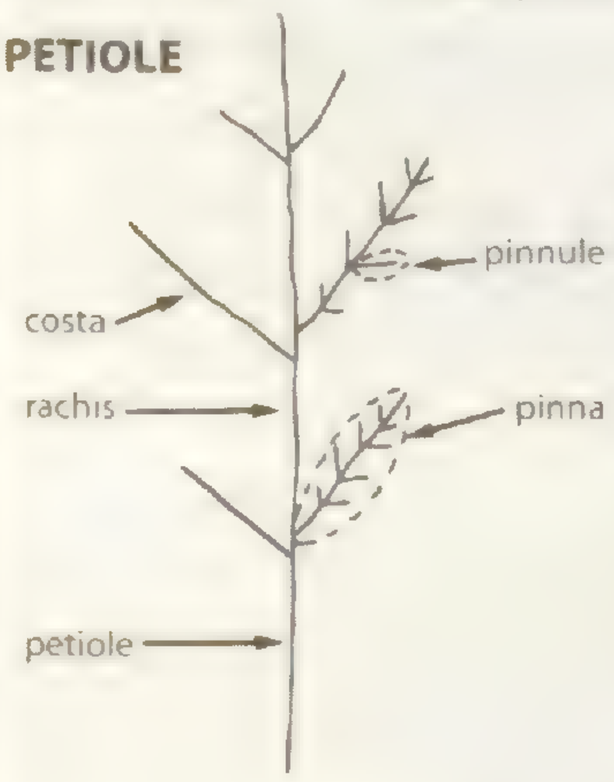
1 pseudoverticillate (count scars / whorl)
2 spiral arrangement (count orthostiches)

CROWN

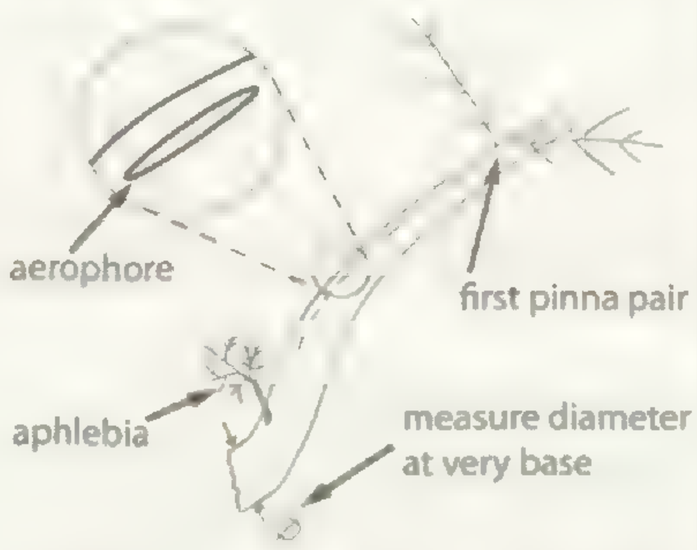


try to sketch habit of plant

PETIOLE



aerophores: brown to white elongate patches of soft tissue allowing air to enter in the petiole and situated in one to several more or less regular rows on either side of the petiole



Aphlebia: usually small sized highly dissected pinnae at the base of the petiole with the lamina extremely reduced so that only the veins remain present

Aphlebioid pinnae: like aphlebia, but with conspicuous lamina remnants

If present, ususally located at the very base **of the petiole and difficult to detach,** especially in petioles with long sigmoid base. Often break away in herbarium **specimens. Hence, note abundance, size** and morphology.

LAMINA

texture: herbaceous, subcoriaceous, coriaceous

colour: usually different shades of green to glaucous: note whether surfaces are dull or shiny

First (basal) pair of pinna is **reflexed**, if costae directed towards petiole base (+/- acute angle with petiole).

This often applies to basal pinnae, but may also be characteristic for pinnules and segments and should then be documented likewise.

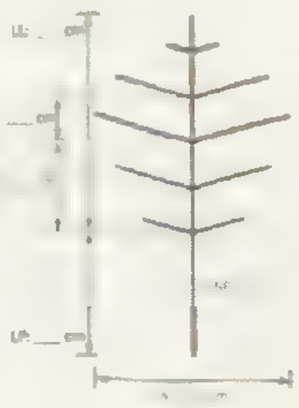
COMPOSITION OF COLLECTION

1. Petiole **base** to first pair of pinna (petiole may be halved in very large fronds), fold several times if necessary.
2. One or sevaral **pinnae** (according to size) from widest part of lamina. Include rachis fragment. Pinna on one side of rachis may be pruned away. If dimorphic, collect fertile and sterile pinnae from respective frond parts.
3. **Apex** of frond.
4. Scales from petiole base in **enveloppe, if caducous.**
5. Mould of trunk surface if petiole **scars not obscured by persistent petiole bases.**
6. Leaf fragment in silicagel.
7. Adventitious buds, some adventitious **roots for anatomical** studies, croziers for anatomical **studies of young scales may** be helpful.

One frond yields one specimen. Very large fronds may yield two specimens. Then the petiole should be halved and a frond apex for the second specimen should be collected from another frond of the same plant.

If possible, take at least **three photos.**

1. Habit of the plant (especially the crown)
2. Zoom in on the stem apex
3. A small part ot the trunk at breast height.



Measurements: This is a schematic representation of a tree fern frond. Measurements should be taken in cm.

LP: length of petiole, measured from base of petiole to first pair of pinnae (disregarding aphlebia and aphlebioid pinnae)

LL: length of lamina, measured from first (basal) pair of pinnae to apex of frond

1L: first to longest pinna, measured from first (basal) pair of pinna to the longest pinna (the widest part of the frond)

LF: longest to following pinna, measured from the pinna pair at the widest part of the frond to the next pinna pair

W: width of frond, measured at the widest part of the lamina; measure in a right angle from the rachis and do not straighten out the longest pinnae

NP: count the number of pinna pairs per frond; if the frond has a pinnatifid apex, stop counting as soon as pinna morphology changes significantly; if the frond has a gradually reduced base transforming into aphlebioid pinnae, stop counting as soon as pinna morphology changes significantly.

Figure 7. Reference sheet. This is a synoptic presentation of essential terminology for tree fern description and the composition of a complete specimen. It is recommended that this reference sheet be taken into the field.

and so are unlikely to be used in existing identification keys. Collectors would do valuable service to future monographers by recording field observations on those characters not preserved in the herbarium specimen. A standardized data sheet (Fig. 6) is provided as an example. This sheet may be photocopied and taken along in the field in sufficient number to be able to fill one sheet per tree fern collection (a pdf file for printing can be obtained from the author upon request). The data sheet can be linked to the field book entry under the relevant locality and habitat information via the collection number. Alternatively, the sheet may serve as a memory aid. Either way, the recorded information should be included on herbarium labels, preferably transformed into a short descriptive text. A reference sheet containing an explanation of essential vocabulary and summarizing the way to collect tree fern specimens is provided (Fig. 7).

ACKNOWLEDGEMENTS

I would like to thank France Rakotondrainibe, Harald Schneider, Nobuhira Kurosaki, Marcus Lehnert, Sachiko Nishida, Hery Lisy Ranarijaona, Emile Randrianjohany, and Germinal Rouhan for valuable discussions and support during fieldwork, respectively. Financial support from the National Geographic Society (#7702-40 to F. Rakotondrainibe) and the Muséum National d'Histoire Naturelle de Paris (PPF "Etat et structure phylogénétique de la biodiversité actuelle et fossile") is greatly acknowledged. Furthermore I thank the Association Nationale pour la Gestion des Aires Protégées and the Direction des Eaux et Forêts de Madagascar for granting collecting permits. Fieldwork in Madagascar benefitted from collaboration with the Centre National pour la Recherche sur l'Environnement, Missouri Botanical Garden, Wildlife Conservation Society, Madagascar Fauna Group and many devoted local collaborators. I thank one anonymous reviewer for comments that helped to improve this manuscript.

REFERENCES

BRIDSON, D. & FORMAN, L. (eds.) 2004. The Herbarium Handbook. Royal Botanic Gardens, Kew.

BROOKS, T.M., MITTERMEIER, R.A., MITTERMEIER, C.G., DA FONSECA, G.A. B., RYLANDS, A.B., KONSTANT, W.R., FLICK, P., PILGRIM, J., OLDFIELD, S., MAGIN, G. & HILTON-TAYLOR, C. 2002. Habitat loss and extinction in the hotspots of biodiversity. Conserv. Biol. 16:909-923.

BROWN, K.A. & GUREVITCH, J. 2004. Long-term impacts of logging on forest diversity in Madagascar. Proc. Natl. Acad. Sci, U.S.A. 101: 6045-6049.

BROWNSEY, P.J. 1985. A plea for better collecting and curation of large ferns. Newslett. Austral. Syst. Bot. Soc. 43: 17-19.

CROFT, J. 1999. A guide to collecting herbarium specimens of ferns and their allies. http://www.anbg.gov.au/fern/collecting.html [last accessed 02/09/2005]

DECKART, M. 1959. Abdrucke von Pflanzenteilen nach dem "ROX-Replica"-Verfahren. Mikrokosmos 48: 313-316.

DU PUY, D.J. & MOAT, J. 1998. Vegetation mapping and classification in Madagascar (using GIS): implications and recommendations for the conservation of biodiversity. *in* HUXLEY, C.R., LOCK, J.M. & CUTLER, D.F. (eds.) Chorology, taxonomy, and ecology of the African and madagascan floras. Royal Botanic Gardens, Kew.

FEIX, G. & HOWORKA, H. 1965. Zur Verwendung von kalthärtendem Silikonkautschuk als Abformmaterial in der Kriminalistik. Wiss. Z. Humboldt-Univ. Berlin, Math.-Naturwiss. Reihe 14: 451-460.

GREEN, P.B., HAVELANGE, A. & BERNIER, G. 1991. Floral morphogenesis in Anagallis: Scaning-electron-micrograph sequences from individual growing meristems before, during, and after the transition to flowering. Planta 185: 502-512.

HERNÁNDEZ, L.F., HAVELANGE, A., BERNIER, G. & GREEN, P.B. 1991. Growth behavior of single epidermal cells during flower formation: Sequential scanning electron micrographs provide kinematic patterns for *Anagallis*. Planta 185:139-147.

HICKS, A.J. & HICKS, P.H. 1978. A selected bibliography of plant collection and herbarium curation. Taxon 27:63-99.

HOLTTUM, R.E. 1957a. Instructions for collecting tree ferns. Fl. Males. Bull. 13: 567.

HOLTTUM, R.E. 1957b. The scales of the Cyatheaceae. Kew Bull. 12: 41-45.

INGRAM, J.C. & DAWSON, T.P. 2005. Inter-annual analysis of deforestation hotspots in Madagascar from high temporal resolution satellite observations. Int. J. Remote Sensing 26: 1447-1461.

ISAAC, N.J.B., MALLET, J. & MACE, G. M. 2004. Taxonomic inflation: its influence on macroecology and conservation. Trends Ecol. Evol. 19:464-469.

JOHNS, R.J. 2000. Proforma for data collection and description of tree ferns. http://www.rbgkew.org.uk/scihort/forms/treefern-proforma.doc [last accessed 02/09/2006]

JÖRG, F. 1965. Mikroskopische Abdruckverfahren. Methoden zur zerstörungsfreien Oberflächenprüfung biologischen und technischen Materials. Das Tyloseverfahren. Mikrokosmos 54: 257-263.

KOECHLIN, J., GUILLAUMET, J.L. & MORAT, P. 1974. Flore et végétation de Madagascar. Kramer, Vaduz.

KORALL, P., PRYER, K.M., METZGAR, J., SCHNEIDER, H. & CONANT, D.S. (2006). Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. Molec. Phylogen. Evol. 39: 830-845.

KUBITZKI, K. 1990. Families and Genera of Vascular Plants. Vol. 1, Pteridophytes and Gymnosperms. KRAMER, K.U. & GREEN, P.S. (vol. eds.). Springer, Berlin.

KWIATKOWSKA, D. & DUMAIS, J. 2003. Growth and morphogenesis at the vegetative shoot apex of *Anagallis arvensis* L. J. Exp. Bot. 54: 1585-1595.

LARGE, M.F. & BRAGGINS, J.E. 2004. Tree ferns. Timber Press. Portland, Cambridge.

LIESNER, R. & al. 2006. Field techniques used by Missouri Botanical Garden. Available online at http://www.mobot.org/MOBOT/molib/fieldtechbook/handbook.pdf [last accessed 05/01/2006]

LOSKE, T. 1959. Methoden der praktischen Textilmikroskopie. II. Mikroskopische Verfahren für verarbeitete textile Halb- und Fertigfabrikate. Mikrokosmos 48: 161-168.

MACE, G.M. 2004. The role of taxonomy in species conservation. Philos. Trans., Ser. B 359:711-719.

McNEELY, J.A. 2002. The role of taxonomy in conserving biodiversity. J. Nat. Conserv. 10:145-153.

MORAN, R.C. 2004. A natural history of ferns. pp. 140-146. Timber Press, Portland, Cambridge.

MORTEMORE, R.A. 1968. Making rubber duplicates of mushrooms. Michigan Bot. 7: 184-187.

OLDFIELD, D. & SYMES, T. 1996. Long term natural ageing of silicone elastomers.

Polymer Testing 15:115-128.
PRYER, K.M., SCHUETTPELZ, E., WOLF, P.G., SCHNEIDER, H., SMITH, A.R. & CRANFILL, R.B. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. Amer. J. Bot. 91:1582-1598.
RANARIJAONA, H.L.T. 1993. L'exploitation des fougères arborescentes (Cyathéacées) du Parc National de Ranomafana: Taxonomie et biologie des espèces, aspects socio-économique. 93 pp. Diplôme d'Etudes Approfondies en Sciences Biologiques Appliquées, option Ecologie Végétale. Université d'Antananarivo, Antananarivo.
RAKOTONDRAINIBE, F. 2003. Diversity, ecology, and distribution of the pteridophyte flora. *in* GOODMAN, S.M. & BENSTEAD J.P. (eds.) Natural history of Madagascar. The University of Chicago Press, Chicago.
ROUX, J.P. 2001. Conspectus of Southern African pteridophyta. Southern African Botanical Diversity Network Report No. 13, pp. 221-222. SABONET, Pretoria.
SCHATZ, G.E. 2002. Taxonomy and herbaria in service of plant conservation: Lessons from Madagascar's endemic families. Ann. Missouri Bot. Gard. 89: 145-152.
SHEFFIELD, E., CUTTER, E.G., DOUGLAS, G.E. & LINDSAY, S. 1991. High-resolution scanning electron-microscopy of fern gametophytes - applications of a nondestructive method. Amer. Fern J. 81:128-133.
STOLZE, R.G. 1973. Inadequacies in herbarium specimens of large ferns. Amer. Fern J. 63: 25-27.
TIWARI, S.C. & GREEN, P.B. 1991. Shoot Initiation on a *Graptopetalum* Leaf - Sequential Scanning Electron-Microscopic Analysis for Epidermal Division Patterns and Quantitation of Surface Growth (Kinematics). Canad. J. Bot. 69: 2302-2319.
WILLIAMS, M.H. & GREEN, P.B. 1988. Sequential scanning electron microscopy of a growing plant meristem. Protoplasma 147: 77-79.
WILLIS, F., MOAT, J. & PATON, A. 2003. Defining a role for herbarium data in Red List assessments: a case study of *Plectranthus* from eastern and southern tropical Africa. Biodiv. Conserv. 12:1537-1552.

BOOK REVIEW

A REVISION OF RADDI'S PTERIDOLOGICAL COLLECTION FROM BRAZIL (1817--1818). Pichi Sermolli, R.E.G. & Bizzarri, M.P. 2005. Webbia 60(1): I--VII, 1--393. ISSN 0083--7792. Available as a back issue, price 60 Euros (per volume). Paperback.

This is an important issue of *Webbia*, containing detailed information about Raddi's pteridological collections from Brazil made during 1817 to 1818. It is a commemorative issue of Webbia's centenary. Unfortunately, the senior author of this paper died before its publication. Most of the annotations are attributed to him, but the account of *Lycopodium* is by the second author. This is a timely and extremely useful bibliography for anyone engaged in ferns, especially in Brazilian ones. Unfortunately, the price of the book is extremely high.

The presentation of this paper is a mixture of contents of a book and a regular paper in a periodical. It comprises a special and commemorative cover (with an interesting drawing of a fiddlehead in the front-cover and a beautiful colored illustration of the Mt. Corcovado in the back-cover), followed by a Presentation, Preface, Introduction (divided in 14 topics and 42 notes), Bibliographical References, Acknowledgements, and two Indices (Index to Names/New Species, New Combinations and New Names; Index). The last Index, in fact, is a list of contents of the book. It would have been beneficial to have provided a small abstract, an index of the illustrations, and moved the list of contents to the initial pages of the book.

The Introduction includes a discussion of Raddi`s life and activities. Here the reader can find information about the precise dates of Raddi's itinerary in Brazil, method of working used by him in the papers (Raddi 1819, 1825) about these collections, data about the original labels on the material, images of his handwriting, and herbaria where he deposited his specimens, many of which serve as types. According to the authors, the complete and original set of Raddi's pteridological collection from Brazil, including several types, is at the general herbarium of Pisa (PI) and in excellent conditions. A second set of this collection is preserved in the *Herbarium Universitatis Florentinae* (FI). The authors called attention to the fact that this second set was included by Parlatore in the FI herbarium only 13 years after Raddi's death. Consequently, these specimens at FI cannot be regarded in any way as the holotypes of Raddi's new species. Only some sheets at FI were considered as isotypes. Other duplicates of the Raddi's original pteridological collection were distributed to European herbaria such as B, BOLO, BM, BR, K, P, and PRC. Details about these duplicates at K, BM, P and BR herbaria are given in this paper. In the topic "number 11" of the Introduction the authors provide details about the procedures followed by them for presenting the new typification of Raddi's taxa in this paper. On the topics 12, 13, and 14 of the Introduction, they present important comments about Raddi's manuscripts and illustrations. All of the 42 notes presented at the end of the Introduction add more details to the main text of the introduction. The topic 14 and the note [40] explain the sequence of presentation of the study made by the authors. Basically, the results are presented following the same sequence of genera and species adopted in the second book published by Raddi (Raddi 1825 – *Plantarum brasiliensium nova genera et species novae vel cognitae. Pars I (Filices)*). The first studied group is *Salvinia*; *Lycopodium* is the last one.

The results show that Raddi recognized 30 genera,148 species, and 9 infraspecifc taxa. The present authors, however, recognized these taxa in 67 genera, 152 species, and five varieties..

Each species is provided a full name and reference adopted by Raddi in his book of 1825 (and in 1819 for some taxa), numbers appear on both extremities of the page at the same line of the name (at left side: the genus-species number; at right side: the number of the figure). This figure when presented represents the holotype or lectotype of the name. Below the Raddi's name appears the accepted name (in bold and italic) for the taxon in the opinion of the authors, followed by some observations on the original collection at PI Herbarium. After it, there is a topic called Annotations. In this part the authors present all comments about the taxon, including its typification and similarities and differences with other taxa, as well as data on the geographical distribution in Brazil and other countries. Complementary notes are also present in this part of the book and sometimes are too big for reading. The illustrations are excellent black and white photographs from the specimens at PI Herbarium. Allied to the text they are an important and useful guide to understand Raddi's species. Unfortunately, for some species there is no illustration.

The following nomenclatural novelties appeared in the book (all ascribed to the senior author): 1) A new species *Doryopteris pentagona* Pic.Serm. 2) A new name for *Polypodium laetum* Raddi (= *Goniophlebium sehnemii* Pic.Serm.), and 3) 12 new combinations: *Amauropelta* subgen. *Uncinella* (A.R. Sm.) Pic.Serm., *Amauropelta aspidioides* (Willd.) Pic.Serm., *A. linkiana* (C. Presl) Pic.Serm., *A. ptarmica* (Kunze ex Mett.) Pic.Serm., *A. retusa* (Sw.) Pic.Serm., *Antigramma balansae* (Baker) Pic.Serm., *Campyloneurum pentaphyllum* (Willd.) Pic.Serm., *Christella deversa* (Kunze) Pic.Serm., *C. raddii* (Desv.) Pic.Serm., *Lomariopsis fraxinifolia* (Raddi) Pic.Serm., *Pecluma schkuhrii* (Raddi) Pic.Serm., and *Sphaerocionium venustum* (Desv.) Pic.Serm.

Several recently published works on neotropical ferns were missed by the authors during preparation of this work. As a result, some of the new combinations proposed are superfluous. For example, the combination *Antigramma balansae* (Baker) Pic.Serm was published three years before by Sylvestre & Windisch (2002). Also, as pointed out by Moran (2000), *Lomaria fraxiinifolia* Raddi is a taxonomic synonym of *L. marginata* (Schrad.) Kuhn. If one accepts this synonymy, then the new combination made in the book (*Lomariopsis fraxinifolia* (Raddi) Pic.Serm.) is unnecessary.

In the Pichi Sermolli`s opinion *Adiantum brasiliense* Raddi (1825) is a good species and quite different from *A. abscissum* Schrad. (1824). However, he did not explain details about *A. abscissum*. Pichi Sermolli pointed out that the two original specimens of *A. brasiliense* at PI are different between them. He numbered the first specimen as 1 (inside a circle) and commented that it is an anomalous form of the species (especially because the pinnules showed different forms), whereas the second specimen (numbered as 2) represents the regular form of the species and it was illustrated by Raddi. He designated the specimen 2 as the lectotype. He also commented that *A. abscissum* could be a synonym of *A. curvatum* Kaulf. However, Prado (2003), not cited by Pichi Sermolli, provided a key for the species of *A. trapeziforme* group in Brazil and *A. abscissum* and *A. curvatum* appeared as a good species rather than *A. brasiliense*. The characters used by Prado (2003) to recognize species in the group were based on the indument of the frond (hairs and scales on the rachis and/or indusia), while Pichi Sermolli considered the frond size and form of the pinnules, two characters quite variable in the genus. Certainly, similar cases involving other species and genera appear

in the book and also they will appear commented in the forthcoming literature.

Despite the comments noted above, this is a valuable contribution to the study of Brazilian ferns and it is an obligatory reference for any future work on pteridophytes from Brazil. The authors are to be congratulated on its impressive compilation.

REFERENCES

MORAN, R.C. 2000. Monograph of the neotropical species of *Lomariopsis* (Lomariopsidaceae). Brittonia 52: 55--111.

PRADO, J. 2003. New species in *Adiantum* from Brazil. American Fern Journal 91(4): 76--80.

RADDI, G. 1819. Synopsis filicum brasiliensium auctore Josepho Raddio ex XLviris Societatis Italicae Scientiarum aliarumque Academiarum Socio. Pp. 1--19. tav. 1--2. Bononiae (Typ. Annesii de Nobilibus). [seors. Prae-impr. Ex Opusc. Sci. 3(5): 279--297. tav. XI--XII. 1819].

RADDI, G. 1825. Plantarum brasiliensium nova genera et species novae vel minus cognitae collegit et descripsit Josephus Raddius ex XL. Viris Societatis Italicae Scientiarum, Academiarum Georgophilorum, Helveticae, Linneanae, et Philomathicae Paris: aliarumque sodalis. Pars I. (Filices). Florentiae.

SYLVESTRE, L.S. & WINDISCH, P.G. 2002. New combinations in *Antigramma* C. Presl (Aspleniaceae) and a synopsis of the species. Bradea 22(1): 3--7.

J. Prado

INSTRUCTIONS FOR AUTHORS

PAPERS should not usually exceed 20 printed pages and are generally expected to be considerably shorter. Review articles, as well as reports of original research, are encouraged. Short notes are acceptable e.g. new records. The senior author should supply a fax and email address to facilitate correspondence.

MANUSCRIPTS should be submitted in English (British) in electronic format (preferably) or hard copy (two copies), in 10-point Times New Roman font and double spaced. Electronic versions of text and tables should be compatible with WORD, with figures as pdf or jpg files, and sent as email attachments or CDroms. All manuscripts will be refereed

THE TITLE should reflect the content of the paper and be in BOLD CAPITALS (11-point) and centrally aligned. Generic and specific names should be in italics and any title containing a generic or specific name must be followed by the family and Pteridophyta in brackets e.g.

***TRICHOMANES SPECIOSUM* (HYMENOPHYLLACEAE: PTERIDOPHYTA) IN SOUTHERN SPAIN**

AUTHOR ABBREVIATIONS should follow Pichi Sermolli's (1996) Authors of scientific names in Pteridophyta, Royal Botanic Gardens, Kew.
MAIN HEADINGS: should be in **BOLD CAPITALS** (10-point) and centrally aligned.
SUBSIDIARY HEADINGS: should be in **bold**, the first letter of each word in capitals, the rest in lower case and left-aligned.
AUTHORS' NAMES AND FULL ADDRESSES: follow the title and are centrally aligned.
KEY WORDS: up to ten.
ABSTRACT: should reflect the content of the paper.
FIGURES: there is no distinction between photographs and line drawings in numbering. All should be presented in a form ready for reproduction, ideally in JPG format (please contact editor with queries), with a scale bar where appropriate. Lettering or numbers (Arabic) should be in the bottom left using uppercase Times Roman and be sufficiently large to be legible if reduction is necessary during printing. The number of photographs allowed in any one issue is limited by cost. Figure captions should be on a separate sheet.
TABLES: can be printed in either portrait or landscape format. Authors should consider this when preparing tables. Authors should ensure that tables fit the printed page size in a legible form.
MEASUREMENTS: should follow the metric system.
CHECKLISTS: should follow the format of Baksh-Comeau, Fern Gaz. 16(1, 2): 11-122.
REFERENCES: should follow the style of a recent issue of The Fern Gazette, e.g.:-

HOOKER, W.J. 1864. Species Filicum, 5. Dulau & Co., London.
MORTON, C.V. 1947. The American species of *Hymenophyllum*, section *Sphaeroconium*. Contr. U.S. Natl. Herb. 29(3): 139-201.
STEVENSON, D.W. & LOCONTE, H. 1996. Ordinal and familial relationships of pteridophyte genera. In: CAMUS, J.M., GIBBY, M. & JOHNS, R.J. (Eds) Pteridology in perspective, pp. 435-467. Royal Botanic Gardens, Kew.

JOURNAL ABBREVIATIONS: should follow Botanico Periodicum Huntianum & Supplements.
Alterations from the original text at proof stage will be charged for unless they are minor points of detail. Twenty-five offprints will be supplied free to the senior author.

Missouri Botanical Garden Library
3 1753 00339 9117

THE BRITISH PTERIDOLOGICAL Soc

Registered Charity No. 1092399

Patron: **HRH The Prince of Wales**

Officers and Committee from March 2006

President: **Dr A.F. Dyer,** 499 Lanark Road West, Balerno, Edinburgh EH14 7AL
E-mail: President@eBPS.org.uk

Vice-Presidents: **M.H. Rickard, Prof. B.A. Thomas**

Honorary General Secretary: **Dr Y.C. Golding**, 7 Grange Road, Buxton, Derbyshire SK17 6NH
E-mail: Secretary@eBPS.org.uk

Treasurer: **Mrs G. Smith**, Rookwood, 1 Prospect Road, Oulton Broad, Lowestoft, Suffolk NR32 3PT; E-mail: Treasurer@eBPS.org.uk

Membership Secretary: **M.S. Porter**, 5 West Avenue, Wigton, Cumbria CA7 9LG
E-mail: Membership@eBPS.org.uk

Meetings Secretary: **P.J. Acock**, 13 Star Lane, St Mary Cray, Kent BR5 3LJ
E-mail: Meetings@eBPS.org.uk

Conservation Officer: **Dr H.S. McHaffie**, 180 Granton Road, Edinburgh EH5 1AH
E-mail: Conservation@eBPS.org.uk

Conservation Officer: & Recorder **Dr F.J. Rumsey**, Dept. of Botany, The Natural History Museum, Cromwell Road, London SW7 5BD
E-mail: Conservation@eBPS.org.uk/Recorder@eBPS.org.uk

Editor of the Bulletin: **Miss A.M. Paul**, Dept. of Botany, The Natural History Museum, Cromwell Road, London SW7 5BD; E-mail: Bulletin@eBPS.org.uk

Editor of The Fern Gazette: **Prof. M. Gibby**, Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR; E-mail: FernGazette@eBPS.org.uk

Editor of Pteridologist: **Dr J.W. Merryweather**, 'The Whins', Auchtertyre, by Kyle of Lochalsh, Wester Ross IV40 8EG; E-mail: Pteridologist@eBPS.org.uk

*Editor of BPS WWW Site – **www.eBPS.org.uk**:* **A.C. Pigott**, Kersey's Farm, Mendlesham, Stowmarket, Suffolk IP14 5RB; E-mail: Webmaster@eBPS.org.uk

Elected Committee Members: **R.G. Ackers, A.R. Busby, Dr M. Hayward, Dr S.D. Martinelli, F. McGavigan, B.D. Smith, R.W. Sykes**

Booksales Organiser: **Mr F. Katzer,** 13 Hawdene, Broughton, Biggar, ML12 6FW
E-mail: Booksales@eBPS.org.uk

Horticultural Information Officer & Archivist: **A.R. Busby**, 16 Kirby Corner Road, Canley, Coventry CV4 8GD
E-mail: HorticulturalInformation@eBPS.org.uk / Archivist@eBPS.org.uk

Merchandise Organisers: **Mr B.D. & Mrs G. Smith**, Rookwood, 1 Prospect Road, Oulton Broad, Lowestoft, Suffolk NR32 3PT; E-mail: Merchandise@eBPS.org.uk

Plant Exchange Organiser: **Mr J.P. Crowe**, Kellys Cottage, Tredilion, Abergavenny, Gwent NP7 8BB; E-mail: PlantExchange@eBPS.org.uk

Spore Exchange Organisers: **Mr B. & Mrs A. Wright**, 130 Prince Rupert Drive, Tockwith, York YO26 7PU; E-mail: Spores@eBPS.org.uk

Trustees of Greenfield & Centenary Funds: **Dr A.F. Dyer, Dr Y.C. Golding, Mrs G. Smith**

The BRITISH PTERIDOLOGICAL SOCIETY was founded in 1891 and today continues as a focus for fern enthusiasts. It provides a wide range of information about ferns through the medium of its publications and other literature. It also organises formal talks, informal discussions, field meetings, garden visits, plant exchanges, a spore exchange scheme and fern book sales. The Society has a wide membership that includes gardeners, nurserymen and botanists, both amateur and professional. The Society's journals, *The Fern Gazette*, *Pteridologist* and *Bulletin*, are published annually. *The Fern Gazette* publishes matter chiefly of specialist interest on international pteridology, the *Pteridologist*, topics of more general appeal, and the *Bulletin*, Society business and meetings reports. **WWW site**: http://www.eBPS.org.uk

Membership is open to all interested in ferns and fern-allies. **SUBSCRIPTION RATES** (due on 1st January each year) are Full Personal Members £20, Personal Members not receiving *The Fern Gazette* £16, Student Members £10, Subscribing Institutions £33. Family membership in any category is an additional £2. Applications for membership should be sent to the Membership Secretary (address above) from whom further details can be obtained. (Remittances made in currencies other than Sterling are £5 extra to cover bank conversion charges.) **Airmail** postage for all journals is an extra £4, or for those not receiving *The Fern Gazette* £2.50. **Standing Order** forms are available from the Membership Secretary and the BPS website.

Back numbers of *The Fern Gazette*, *Pteridologist* and *Bulletin* are available for purchase from P.J. Acock, 13 Star Lane, St Mary Cray, Kent BR5 3LJ; E-mail: BackNumbers@eBPS.org.uk

THE FERN GAZETTE

VOLUME 17 PARTS 6, 7 & 8 2006

CONTENTS

MAIN ARTICLES

The cell walls of pteridophytes and other green plants – a review
Z.A. Popper 315-332

***Trichomanes speciosum* (Hymenophyllaceae: Pteridophyta) in northwestern France**
S. Loriot, S. Magnanon & E. Deslandes 333-349

A moulding method to preserve tree fern trunk surfaces including remarks on the composition of tree fern herbarium specimens
T. Jamssen 351-363

BOOK REVIEWS

The first botanical collectors in Nepal – The Fern collections of Hamilton, Gardner and Wallich – lost herbaria, a lost botanist, lost letters and lost books somewhat rediscovered
C.R. Fraser-Jenkins 314,350

A Revision of Raddi's Pteridological Collection from Brazil (1817--1818)
R.E.G Pichi Sermolli & M.P. Bizzarri 364-366

INSTRUCTIONS FOR AUTHORS 368

ISSN 0308-0838